VOLUME 1, NO. 1 FEBRUARY 1960

BIOLOGY LIBRARY
JUN 16 1960

GENETICAL RESEARCH

EDITORIAL BOARD

C. H. WADDINGTON (*Chairman*)
CHARLOTTE AUERBACH D. LEWIS
H. GRÜNEBERG G. PONTECORVO
W. HAYES A. ROBERTSON

EXECUTIVE EDITOR

E. C. R. REEVE

Indiana University
MAY 23 1960
Library

PUBLISHED BY

CAMBRIDGE UNIVERSITY PRESS

Bentley House, 200 Euston Road, London, N.W. 1
American Branch: 32 East 57th Street, New York 22, N.Y.

PRICE 40*s.* net (U.S.A. $7.00)

GENETICAL RESEARCH

GENETICAL RESEARCH publishes original work on all aspects of genetics, or in any field of research which has an important bearing on genetics. Papers will be submitted to referees before acceptance. They will generally be printed in order of acceptance. In addition, short notes limited to 1500 words or the equivalent space including figures, etc., may be included if received 3 months before publication date.

EACH ANNUAL VOLUME will consist of three parts (to be published in February, July and November) plus an index.

SUBSCRIPTIONS may be sent to any bookseller or to the Cambridge University Press, 200 Euston Road, London, N.W. 1. Subscriptions in the U.S.A. should be sent to the American Branch of the Press, 32 East 57th Street, New York 22, N.Y. The subscription price is £5 net (including postage) for an annual volume of three parts ($17.50 in the U.S.A.), payable in advance; Separate parts 40*s*. or $7.00 each (plus postage).

NOTES FOR CONTRIBUTORS

CONTRIBUTIONS will be welcomed from scientists of all nationalities, but must be written in English, with an English summary. An additional summary in French, German, Russian or Italian will be accepted. Papers should be sent to the Executive Editor, Dr. E. C. R. Reeve, Institute of Animal Genetics, West Mains Road, Edinburgh 9.

GENERAL. Submission of a paper or note will be taken to imply that it is unpublished and is not being considered for publication elsewhere. Papers published in *Genetical Research* may not be reprinted or published in translation without permission from the Editorial Board. Papers should be as concise as clarity permits, and illustrations kept as few as possible. Attention to the following instructions will assist rapid publication.

TYPESCRIPTS. A top copy and one carbon copy should be submitted. They should be typed with double-spacing, on one side of the paper, leaving margins of about 1½ inches at the left-hand side and head of each sheet. Quarto is preferred to foolscap paper. Heavily corrected typescripts may have to be returned to the author for retyping. Papers should state the name and address of the author (or his proxy) to whom proofs are to be sent. The address of the laboratory at which the work was carried out will be printed with the authors' names at the head of the paper, and changes of address and sources of financial support may be added in footnotes.

A short title should be supplied for running headlines; this must not exceed 50 characters, counting each letter and space as one character.

(*continued inside back of cover*)

ics, or
rs will
ted in
e equi-
before

ned in

versity
uld be
, N.Y.
ume of
40*s*. or

t must
nary in
to the
Mains

is un-
blished
vithout
ty per-
llowing

. They
nargins
is pre-
eturned
of the
oratory
at the
rt may

exceed

f cover)

Genet. Res., Camb. (1960), **1**, *pp.* 1–24
With 2 text figures
Printed in Great Britain

Localized negative interference and its bearing on models of gene recombination

By R. H. PRITCHARD*
Department of Genetics, The University, Glasgow

(*Received* 25 *April* 1959)

1. INTRODUCTION

The use of techniques which permit automatic selection of rare recombinants in micro-organisms, and extensive use of tetrad analysis, have revealed properties of recombination which were not apparent from earlier studies.

For example, in the course of experiments in which selective techniques were used to establish the linkage relationships of a number of allelic mutants of *Aspergillus nidulans* (Pritchard, 1955) it was found that among recombinants with respect to very closely linked markers, the distribution of other markers on either side of the closely linked pair was of an unexpected type. The data were interpreted in terms of localized negative interference; that is to say, it was supposed that coincidence of recombination between the very closely linked markers and recombination in adjacent intervals occurred with greater than random frequency. The data indicated, on this hypothesis, that a positive correlation between recombination events was restricted to a few tenths of a map unit —i.e. the effect was highly localized—and its occurrence was therefore detected only because the markers used in this study were very closely linked.

That the effect is not confined to *Aspergillus* is suggested by earlier observations apparently of a similar nature in *Neurospora* (Giles, 1951, 1955) and *Drosophila* (Demerec, 1928; and possibly Sturtevant, 1951), and numerous other cases have been described more recently in bacteriophage (Streisinger & Franklin, 1956; Chase & Doermann, 1958), in *Neurospora* (St. Lawrence, 1956; Mitchell, 1956; Freese, 1957*a*, *b*; De Serres, 1958), in *Aspergillus* (Calef, 1957), in yeast (Leupold, 1958), and in *Drosophila* (Chovnick, 1958). The phenomenon seems to be a common one, although the basis for it may not be the same in all cases.

A second feature of recombination which has become apparent from extensive tetrad analysis in yeast (Lindegren, 1955; Roman, 1956; Leupold, 1958), *Neurospora* (Mitchell, 1955*a*, *b*; Case & Giles, 1958) and *Aspergillus* (Strickland, 1958*a*) is the rare occurrence of tetrads with 3 : 1 ratios with respect to one or more loci. The frequency of tetrads of this type, although low, is higher in heterozygotes than in homozygotes, and in some cases an interpretation of their origin on the basis of the known genetic mechanisms listed by Emerson (1956), such as polyploidy, is unsatisfactory. The occurrence of irregularities of this sort has also been inferred

* Present address: Medical Research Council Microbial Genetics Research Unit, Hammersmith, London.

from analysis of half-tetrads following meiosis or mitosis (Roman, 1956; Leupold, 1958; Hexter, 1958). The occurrence of 3 : 1 ratios in tetrads has been attributed to hypothetical mechanisms distinct from crossing over and termed variously gene conversion (Lindegren, 1955), transmutation (Horowitz—see Beadle, 1957), transreplication (Glass, 1957). Roman (1956) uses the term 'non-reciprocal recombination' in connexion with his data: it should be noted that if among the four meiotic products of a cell heterozygous for two linked loci a 3 : 1 ratio occurs with respect to one of them, one of the four products will be a recombinant for which there is no complementary recombinant class.

Two questions arise from these observations. Firstly, do reciprocal recombinants and non-reciprocal recombinants result from two different mechanisms or are they the result of different aspects of a single mechanism of recombination between linked loci? Secondly, how can localized negative interference be interpreted in relation to current models of chromosome replication and recombination? In this connexion it should be pointed out that my interpretation of the data from *Aspergillus* in terms of localized negative interference has been questioned by some authors (Mitchell, 1957). The alternative is that if among a population of recombinants some were the consequence of a reciprocal recombination process (which we will call crossing over) not associated with negative interference and some the consequence of a different process, the non-reciprocal transfer of a marker from one chromosome to its homologue, the distribution of markers on either side of the interval in question might formally be described in terms of negative interference but would be significant only as an indication of the existence of more than one mechanism of recombination. The data already published (Pritchard, 1955; Roper & Pritchard, 1955), however, are difficult to reconcile with an interpretation of this sort.

In the present paper the evidence relating to localized negative interference is reviewed and added to and its implications are considered.

2. MATERIAL AND METHODS

For details of the origin of the mutant strains used in this work and the techniques employed in genetic analysis of *Aspergillus nidulans*, reference should be made to Pontecorvo, Roper, Hemmons, Macdonald & Bufton (1953), Pritchard (1955), Pontecorvo & Käfer (1958) and Käfer (1958).

3. MAP OF THE *ad8* CISTRON

Seven adenine-requiring mutations, all recessive and allelic by the *cis-trans* test, have now been mapped. Each occupies a different site in the cistron (Fig. 1).

The method of calculating the recombination fractions between these alleles has already been published (Pritchard, 1955). It is an indirect one involving a comparison of the number of colonies produced by a suspension of ascospores plated on different selective media at different densities. Two of the seven alleles are phenotypically distinguishable from the other five. Both (*ad16* and *ad20*) permit

Fig. 1. Above: map of the *ad8* cistron showing recombination fractions $\times 10^5$. Below: map of chromosome I showing location of markers referred to in text. Nutritional requirements are *an*, aneurin; *paba*, *p*-amino-benzoic acid; *ad*, adenine; *bi*, biotin; and *y*, yellow conidia. Markers on other chromosomes used were *nic2*, nicotinic acid; *s12*, thiosulphate; *pyro4*, pyridoxin; *w3*, white conidia; and *Acr1*, resistance to acriflavine (see Käfer, 1958). Throughout this paper mutant alleles are referred to without the allele number except in the case of the *ad* alleles. Wild-type alleles are designated by superscript +.

slow growth in the absence of an external supply of adenine, and *ad20* is suppressed by either of two mutant alleles at unlinked loci (*su1ad20* and *su2ad20*). The suppressors are specific for *ad20*.

4. THE OCCURRENCE OF LOCALIZED NEGATIVE INTERFERENCE

From fourteen crosses so far made of the general type y ad_x/ad_y *bi*, the majority of adenine-independent types recovered was associated with recombination between *y* and *bi*. One of the two possible recombinant classes was always in excess of the other, the recombinants being prevalently either *y bi* or y^+ bi^+.

The seven mutant alleles could be assigned a linear order on the basis of the majority recombinant class without any inconsistencies, and the map distances computed from the total frequency of adenine-independent segregants were approximately additive (Fig. 1).

The ability to obtain a map which is qualitatively and quantitatively consistent indicates that the seven sites are linearly arranged, not only with respect to each other, but also with respect to neighbouring loci. The data provide no support for models of the chromosome involving side-chains of the type proposed by Taylor (1957), since these require a branched linkage map. Evidence of this sort against a branched linkage structure has been known for some time, not, as maintained by Freese (1957*a*), only recently from experiments with bacteriophage. Thus, that allelic mutations were separable and linearly arranged with respect to each other, and to the rest of the linkage map, was first demonstrated by Lewis (1945) for alleles of '*Star*' in *Drosophila*, although Lewis did not at that time consider them to be alleles of one gene.

Ample confirmation of this observation has since been obtained from *Drosophila* (e.g. Green & Green, 1949; MacKendrick & Pontecorvo, 1952; Lewis, 1952; Green, 1954) and *Aspergillus* (Roper, 1950; Pritchard, 1955; Calef, 1957).

As to the nature of interallelic recombination, it was shown as early as 1952 by Lewis and later by Green (1954) that recombination between allelic mutants in *Drosophila* is generally reciprocal. This also appeared to be so in the case of recombination between allelic *ad* mutations in *Aspergillus* since it was shown (Roper & Pritchard, 1955) that from diploids heterozygous in *trans* for two *ad* alleles it was possible to recover adenine-independent types still doubly heterozygous, but now in *cis*, as a result of a single reciprocal event.

The unexpected feature of the data from crosses of the general type described was that the frequency of parental combinations *y* bi^+ and y^+ *bi* among adenine-independent segregants was in every case greater than 6%, the approximate frequency expected in the absence of interference. In some cases the frequency of parental combinations was as much as 40%.

The data were consistent with the view that the high frequency of parental combinations was due to a localized excess of multiple recombination events over the number expected from coincidence of independent events. The alternative hypothesis, that there was no negative interference but that recombinants were produced by two different mechanisms, crossing over and gene conversion, in

such a way as to simulate negative interference, is not supported by a comparison of the data from crosses involving different pairs of *ad* mutants.

On this hypothesis, among adenine-independent colonies obtained from crosses of the general type

$$\frac{y \quad ad_x \quad + \quad +}{+ \quad + \quad ad_y \quad bi},$$

those originating by crossing over between ad_x and ad_y would have the constitution $y^+ bi^+$, while those originating by conversion of ad_x and ad_y would have the constitution $y\, bi^+$ and $y^+ bi$ respectively. The frequency of adenine-independent colonies of each type can therefore be used to obtain an estimate of the relative frequency of the three types of event provided a correction is made for the expected occurrence of 6% recombination in the inverval ad_y–*bi* among all adenine-independent types, whatever their origin. A proportion of the crossovers between ad_x and ad_y would consequently have the constitution $y^+ bi$, and a proportion of those adenine-independent types due to conversion of ad_x and ad_y would have the constitution $y\, bi$ and $y^+ bi^+$ respectively. Recombination between y and ad_x can be ignored since its frequency in the absence of interference will be negligible.

The apparent frequency of conversion for each of the seven *ad* alleles was calculated in this way from two crosses, that yielding the lowest and that the highest frequency of adenine-independent types (Table 1).

Table 1. *Apparent frequency of gene conversion of seven different alleles compared in different crosses assuming no interference*

Alleles involved in cross	'Crossover' frequency ($\times 10^6$)	'Conversion' frequency ($\times 10^6$)						
		ad20	*ad16*	*ad19*	*ad12*	*ad11*	*ad8*	*ad10*
ad20 and *ad16*	2·60	0·56	0·82	—	—	—	—	—
ad8 and *ad10*	4·38	—	—	—	—	—	0·91	1·15
ad12 and *ad11*	69·7	—	—	—	5·15	11·5	—	—
ad19 and *ad12*	91·2	—	—	0·99	12·9	—	—	—
ad11 and *ad10*	543	—	—	—	—	41·6	—	140
ad19 and *ad8*	749	—	—	21·4	—	—	204	—
ad12 and *ad8*	922	—	—	—	25·9	—	113	—
ad16 and *ad8*	1080	—	30·0	—	—	—	131	—
ad20 and *ad8*	1260	81·0	—	—	—	—	89·0	—

If gene conversion were a mutational event of the type proposed by Lindegren (1955), its frequency might be expected to be specific for a particular allele, in heterozygous condition with the corresponding wild-type allele, and independent of the frequency of recombination between it and a second allele. The data in Table 1 indicate on the contrary that the apparent rate of conversion for a particular allele may differ by a factor of over 100 in different crosses and, in addition, that there is a correlation between the apparent frequency of conversion and the frequency of crossing over. This confirms the conclusion drawn previously

(Pritchard, 1955), from a comparison of this sort between the crosses available at that time, that the data were inconsistent with the view that directed back-mutation makes a significant contribution to the formation of adenine-independent types.

A similar conclusion has been drawn more recently by Chase & Doermann (1958) from a comparison of this sort between crosses involving different rII mutants of bacteriophage, and by Freese (1957*a*) from a comparison of crosses involving different histidine mutants of *Neurospora*.

The possibility that the rates of both crossing over and gene conversion are dependent on some other condition, such as 'intimate association' between chromosomes (Mitchell, 1956), and that this is responsible for the correlation found, is unlikely in this case since the difference in the total frequency of adenine-independent types from crosses involving different pairs of *ad* alleles is clearly related to their relative locations on the linkage map.

The directed mutation of one allele under the influence of an homologous allele in a heterozygote is not the only conceivable mechanism, distinct from crossing over, which might be responsible for tetrads with 3 : 1 ratios and apparent negative interference in random strand analysis. An alternative possibility is that a segment of the genetic material might be copied twice on one of the two alternative templates in a heterozygote (Lederberg, 1955). A mechanism of this sort is favoured by Roman (1956) to account for non-reciprocal recombination in yeast.

Whether an event of this type occurred in *Aspergillus* with a frequency high enough to simulate negative interference could not easily be determined by random strand analysis; it might be by tetrad analysis. In the present case tetrad analysis of crosses between pairs of *ad* mutants could not easily be undertaken, owing to poor ascospore viability in crosses involving these mutants. The reciprocal products of recombination can nevertheless be recovered if they occur by making use of mitotic recombination in heterozygous diploids (Roper & Pritchard, 1955).

Mitotic analysis has certain limitations which make quantitative treatment of the data unreliable. The chief limitations are: firstly, that only two of the four homologous daughter strands produced at mitosis are recovered and some assumption must be made as to the distribution of these to the two daughter cells; and secondly, that the frequency of mitotic recombination is less than that of meiotic recombination by a factor of about 10^4. This means that the frequency of recombination between alleles at mitosis approaches the spontaneous back-mutation rate of the *ad* mutants used in this work.

The genotypes of adenine-independent diploids obtained from two adenine-dependent diploids heterozygous in *trans* for *ad16* and *ad8* have already been described. Data from a third diploid heterozygous for *ad20* and *ad8* are given in Table 2. The results obtained from the three diploids are summarized in Table 3.

A total of fifty-two adenine-independent diploids was obtained. The genotypes of twelve of these (e.g. types 5 and 6 in Table 2) were consistent with an origin by back-mutation of one or other *ad* allele. The number of diploids of this type was about that expected from the relative frequencies of mitotic recombination (*ca.* 10^{-7}) between, and back-mutation (*ca.* 10^{-8}) of, the *ad* mutants used.

Table 2. *Genotypes of adenine-independent diploid types derived from a diploid heterozygous for* ad20 *and* ad8

Intervals ...	...	**a**	**b**	**c**	**d**		**e**		
Parent diploid	...	*an* / +	*paba* / +	*y* / +	*ad20* / +	+ / *ad8*	+ / *bi*	+ / *nic*	*pyro* / +	*Acr* / +

Recombinant type	Genotype†	Inferred origin	No. obtained
1	*an paba y ad20 ad8 bi* / + + + + + +	Reciprocal products of single exchange in **d**	2
2	*an paba y ad20* + + / + + + + + +	Single exchange in **d**. Reciprocal products not recovered	3
3	*an paba y ad20 ad8* + / + + + + + +	3-strand double in **d** and **e**. Reciprocal products of exchange in **d** recovered	1
4	*an paba y ad20* + *bi* / + + + + + +	3- or 4-strand double* in **d** and **e**. Reciprocal products of neither exchange recovered	1
5	*an paba y ad20* + + / + + + + + *bi*	2- or 3-strand double in **d** and **e**. Reciprocal products of neither exchange recovered. Or mutation of *ad8*	1
6	*an paba y* + + + / + + + + *ad8 bi*	2- or 3-strand double in **c** and **d**. Reciprocal products not recovered. Or mutation of *ad20*	2
7	*an paba y* + + *bi* / + + + + *ad8 bi*	?	1

* Alternatively, this type might conceivably have arisen by a single inexactly reciprocal exchange as illustrated in Fig. 3.

† All remained heterozygous *nic*/+, *pyro*/+ and *Acr*/+.

Table 3. *Summary of types of adenine-independent mitotic recombinant diploids obtained from three diploids heterozygous* ad *in* trans

No. of exchanges	Reciprocal products of exchange between alleles	
	Recovered	Not recovered
One	10	19
Two	2	4
2- or 3-strand double or back-mutation	—	12
Three or more exchanges, or mutation + exchange	—	5
Total	12	40

Recombination was clearly involved in the origin of the remaining forty diploids. The genotypes of twenty-nine of these were those expected following a single reciprocal exchange between *ad* alleles (e.g. types 1 and 2 in Table 2). We should expect to recover both products of the exchange in half these provided the occurrence of a mitotic exchange did not influence the segregation of the chromatids involved. Both products were recovered in only ten of the twenty-nine. The deviation from expectation is not statistically significant. If it is nevertheless real, two possible causes might be (*a*) that recombination at mitosis is in some cases not reciprocal, or (*b*) that the reciprocal products of a mitotic exchange segregate preferentially to different daughter nuclei (a non-disjunctional type of segregation is ruled out since all adenine-independent diploids remained heterozygous at the *paba* locus). Since in all nineteen individuals in which reciprocal products were not recovered the wild-type chromosome with respect to adenine requirement was a recombinant in the interval *y–bi* whereas the other chromosome was not, it appears that only one of the two strands involved in the recombination event was recovered and an explanation of the second type appears the more probable.

Of the remaining eleven adenine-independent diploids, six had genotypes consistent with coincidence of two mitotic exchanges, one between the *ad* alleles and one in a neighbouring interval. Reciprocal products of the mitotic exchange between the *ad* alleles were recovered in two of these and reciprocal products of both exchanges in one.

The remaining five diploids could be accounted for only by coincidence of three or more mitotic exchanges (e.g. type 7 in Table 2). In none of these were both products of an exchange between the *ad* alleles recovered. It is not unreasonable to suppose that one or more non-reciprocal events were involved in the origin of some of these individuals, but a larger sample would be necessary to establish this.

Mitotic analysis, then, indicates that recombination at mitosis between allelic mutants is frequently a reciprocal process, and the data are not inconsistent with the possibility that it is almost always so. It is also clear that localized negative interference operates at mitosis as it has been inferred to do at meiosis. This is apparent even if only those diploids are considered in which the reciprocal products of a mitotic exchange between the *ad* alleles were recovered. In two out of twelve diploids of this type recombination between the *ad* alleles was associated with recombination in an adjacent interval. The occurrence of two doubles in so small a sample should be compared with the data of Pontecorvo & Käfer (1958) which show that coincidence of mitotic recombination in two intervals, when these are long, is exceedingly infrequent as would be expected from the overall incidence of recombination at mitosis.

The occurrence in *Aspergillus* of a mechanism of recombination distinct from crossing over is not, of course, ruled out by the data available at present. What is clear is that such a mechanism will not provide an adequate explanation for localized negative interference.

5. THE PATTERN OF LOCALIZED NEGATIVE INTERFERENCE

(*a*) *Introduction*

Coincidence values greater that unity with respect to two intervals would be observed if the occurrence of recombination in one directly increased the probability of recombination in the second. Alternatively (Weinstein, 1918; Sturtevant, 1955), if the distribution of exchanges between the two intervals was a random one but a population of cells undergoing meiosis was heterogeneous with respect to the total incidence of recombination, coincidence values greater than unity would necesssarily result.

A model of the second type was used to account for localized negative interference in *Aspergillus* (Pritchard, 1955). One of the conditions necessary for recombination to take place was called 'effective pairing'. Effectively paired segments were assumed to be very short and discontinuously, although not necessarily randomly, distributed such that only a small fraction of the genome was effectively paired in any one zygote. In this case coincidence of recombination in two intervals would occur with greater than random frequency if these intervals were short enough and close enough to be frequently included within one effectively paired segment.

The term 'effective pairing' was used to differentiate it from pairing observable cytologically. There need be no connexion between the two, and there probably is none. It has been shown that duplication of DNA may occur long before the onset of the cytological events associated with meiosis (see review of Taylor, 1957). If recombination occurs at the time of DNA replication, effective pairing would also occur before the onset of cytologically observable pairing.

Chase & Doermann (1958) have made use of a similar model to account for localized negative interference in bacteriophage; their 'switch regions' would correspond to regions of effective pairing in *Aspergillus*. Discontinuous pairing has been used to account for negative interference extending over larger sections of the genome in *Escherichia coli* (Rothfels, 1952; Cavalli-Sforza & Jinks, 1956) and *Drosophila* (Sturtevant, 1955). The occurrence of negative interference across an inversion in *Drosophila subobscura* (Spurway & Philip, 1952) may also have the same basis.

(*b*) *Experimental method*

An attempt has been made to determine some of the properties of effective pairing segments, such as their mean length, whether they are randomly distributed along chromosomes, and the recombination frequency per unit length. The cross used is shown in Table 4 and has provided preliminary answers to these questions.

The cross was between two strains, one carrying *ad8* and *ad20*, the other carrying *ad11*. Since *ad20* will grow slowly on minimal medium it was possible to select for recombination between *ad8* and *ad11* and follow the segregation of *ad20* among the selected progeny.

Samples of ascospores from a single suspension were plated on two different media, one lacking biotin and nicotinic acid and the other lacking adenine. Colonies obtained from selection I (Table 4) were used to establish control recombination fractions for intervals **a, b,** and **g**. Control values for intervals **c** and **d** could not be obtained from this cross and values obtained from other crosses were therefore used.

Table 4. *Genotypes of recombinants from a cross involving* ad20, ad11 *and* ad8

					g					
Interval ...		**a**		**b**	**c**	**d**	**e**	**f**		
Cross 1 ...	*an*	*paba*	*y*	*ad20*	+	*ad8*	+	*nic*	*Acr*	
	+	+	+	+	*ad11*	+	*bi*	+	+	

Genotype	Selected types: IIa *ad20 ad11* + *ad8* +* (exchange in **e** but not **d**)		IIb *ad20* + *ad11* + *ad8* +* (exchange in **d** and **e**)		I *bi* + *nic* + (control)	
	Interval	No. of colonies	Interval	No. of colonies	Interval	No. of colonies
an paba y bi	**0**	133	**c**	8		
+ *paba y bi*	**a**	76	**ac**	1		
+ + *y bi*	**b**	20	**bc**	0		
an + *y bi*	**ab**	9	**abc**	1		
+ + + *bi*	**c**	5	**0**	69		
an + + *bi*	**ac**	2	**a**	34		
an paba + *bi*	**bc**	1	**b**	21		
+ *paba* + *bi*	**abc**	1	**ab**	4		
an paba y +	**f**	57	**cf**	1	**0**	232
+ *paba y* +	**af**	24	**acf**	1	**a**	93
+ + *y* +	**bf**	5	**bcf**	0	**b**	26
an + *y* +	**abf**	4	**abcf**	0	**ab**	15
+ + + +	**cf**	0	**f**	14	**g**	9
an + + +	**acf**	2	**af**	12	**ag**	4
an paba + +	**bcf**	0	**bf**	2	**bg**	1
+ *paba* + +	**abcf**	0	**abf**	7	**abg**	2
	Total	339		175		382

* Accurate counts of the number of colonies of each type on a number of plates gave 2804 IIa : 108 IIb or 3·85% IIb.

Colonies obtained from selection II were of two types, slow growers (*ad20*) and normal growers. The former are derived by recombination in interval **e**, and the latter by recombination in both **d** and **e**. From the relative frequency of the two types (Table 4) the recombination fraction in **d** among crossovers in **e** can be directly obtained and compared with the control value (Table 5). Colonies of both types were isolated and classified for the remaining marked loci (Table 4), but the

number of each type fully classified in this way was not proportional to their relative frequencies in selection II. The results are summarized in Table 5.

Comparing first selection IIa with selection I it is apparent that selection for recombination in **e** results in a dramatic increase in the frequency of recombination in intervals **c**, **d**, and **f**. (The difference in length between interval **g** in selection I and **f** in selection II will be very small and a comparison between the two can therefore legitimately be made.) The absence of an increase in interval **b** indicates the degree of localization of negative interference.

Table 5. *Recombination fractions calculated from the data given in Table 4*

					g				
Interval ...		**a**	**b**	**c**	**d**	**e**	**f**		
Cross 1 ...	*an* / +	*paba* / +	*y* / +	*ad20* / +	+ / *ad11*	*ad8* / +	+ / *bi*	*nic* / +	*Acr* / +

	Interval					
Selection	**a**	**b**	**c**	**d**	**e**	**f** or **g**
I. *bi* + *nic* +	29·8 ± 2·34	11·5 ± 1·60	*0·089*	*0·055*	0·25	4·55 ± 0·91*
II. *ad11* + *ad8* +	—	—	—	3·85 ± 0·36	—	—
IIa. *ad20* (exchange in **e** but not **d**)	34·8 ± 2·59	11·8 ± 1·75	3·24 ± 0·96	—	—	27·1 ± 2·37
IIb. *ad20* + (exchange in **d** and **e**)	34·3 ± 3·60	20·0 ± 3·02	6·86 ± 1·91	—	—	21·1 ± 3·09
IIa and IIb weighted and pooled data	34·8	12·1	3·38	3·85	—	26·9

Values in italics were obtained from other crosses.

* This fraction is based on data from 382 colonies shown in Table 4 and a further 145 classified as to *y* or *y* + only.

(*c*) *The distribution of effective pairing segments*

The possibility had been considered that effective pairing segments might correspond in position and length with the functional units (or cistrons) of a chromosome. Such a situation would provide circumstantial evidence for a physical discontinuity along chromosomes corresponding to the functional discontinuity. If this were the case, the increase in the recombination fractions in **c** and **f** over the control values should be the same in selections IIa and IIb. This would not be the case if pairing segments were randomly distributed. In this case selection for an exchange in both **d** and **e** would sample a population in which effective pairing segments would have a mean position proximal to that of a similar population of segments sampled by selection for an exchange in **e** and not in **d**. The recombination fraction in interval **c** should therefore be greater in the former

Table 6. *Three crosses, involving different pairs of alleles, showing non-random distribution of additional exchanges on either side of a selected exchange*

Cross 2 ...

	a	b	c	d	e		
	an	*paba*	+	*ad16*	+	*bi*	*pyro* / +	+ / *s12*
	+	+	*y*	+	*ad19*	+	

Selection	Interval a	b	c	d	e or f
I. *pyro* + *s12* +	30·9 ± 3·43	12·7 ± 2·47	*0·048*	—	7·73 ± 1·99
II. *ad16* + *ad19* +			24·6 ± 2·08	0·013	
IIa. *y* (exchange in **d** but not **c**)	37·0 ± 3·55	14·7 ± 2·60	—	—	16·3 ± 2·72
IIb. *y* + (exchange in **d** and **c**)	37·2 ± 4·39	17·3 ± 3·44	—	—	9·92 ± 2·72

Cross 3 ... ...

	b	c	d	e		
	paba	*y*	+	*ad11*	+	*pyro* / +
	+	+	*ad19*	+	*bi*	

Selection	Interval b	c	d	e or f
I. *pyro* + *bi* +	12·6 ± 2·57	?	—	3·43 ± 0·77
II. *ad19* + *ad11* +	—	14·6 ± 1·57	0·015	—
IIa. *y* (exchange in **d** but not **c**)	11·9 ± 2·64	—	—	23·7 ± 3·48
IIb. *y* + (exchange in **d** and **c**)	15·5 ± 4·29	—	—	9·86 ± 3·54

Cross 4 ... ...

	b	c	d	e		
	paba	*y*	*ad20*	+	+	+ / *pyro*
	+	+	+	*ad11*	*bi*	

Selection	Interval b	c	d	e or f
I. *paba* + *pyro* +	10·1 ± 1·64	?	—	8·04 ± 1·48
II. *ad20* + *ad11* +	—	15·7 ± 1·36	0·055	—
IIa. *y* + (exchange in **d** but not **c**)	12·4 ± 2·06	—	—	12·4 ± 2·06
IIb. *y* (exchange in **d** and **c**)	23·3 ± 6·44	—	—	4·65 ± 3·21

population than in the latter. In interval **f**, the reverse should be true—the recombination fraction should be less in the former selection than in the latter.

The results are in agreement with the second alternative. Thus the recombination fraction in **c** is significantly greater in selection IIb than in IIa while the recombination fraction in **f** shows the reverse relationship, although in this case the difference is not significant. In addition, it is apparent that in selection IIb the region of negative interference is extended into interval **b** since the recombination fraction in this interval is significantly greater in IIb than in IIa although that in IIa does not differ from the control value.

In order to see if the same type of correlation could consistently be obtained, similar selections were made in three other crosses. The data are given in Table 6. These crosses involved only two alleles. Recombination fractions in intervals **b** and **e** were compared among crossovers in **d** and among crossovers in both **c** and **d**. The results were in each cross similar to those obtained from cross 1; shifting the point of selection towards *y* increases the recombination fraction proximal to *y* and decreases it in the interval distal to the *ad8* region.

Stated another way, the data from four crosses indicate that among recombinants between very closely linked markers the distribution of correlated exchanges on either side of the selected exchange is not a random one. There is a positive correlation (or negative interference) between additional exchanges in adjacent intervals on the same side of the selected exchange, and a negative correlation (or positive interference) between intervals on different sides of the selected exchange.

It is not necessary to assume that the distribution of exchanges within effective pairing segments is other than a random one to account for these correlations. Indeed, it would be difficult to explain them on the basis of a non-random distribution of exchange events. Rather it seems simpler to assume that they result from variability in the position of these segments.

(*d*) *The length of effective pairing segments*

Data at present available are not sufficiently precise to determine whether effective pairing segments are variable in length. From an analysis of localized negative interference in a different part of the map of *Aspergillus nidulans*, Calef (1957) concluded that effective pairing segments were variable in length and suggested that a proportion of them were very long (at least 15 map units). In cross 1 (Table 5) and in cross 2 (Table 6) the effect of selection for an exchange in the *ad8* cistron on the recombination fraction in the interval *an–paba* (15 map units away) could be followed. In both crosses selection for an exchange in the *ad8* cistron resulted in an increase in the recombination frequency between *an* and *paba*. The increase was in neither case statistically significant, but the trend was sufficiently consistent to suggest that it was a real one.

For two reasons it seems unlikely that this increase can be accounted for by extreme variability in length of effective pairing segments such that a proportion of them extends over a large fraction of the length of chromosome I. Firstly, the

increase in the recombination frequency in the interval *an–paba* in selection IIa compared with the control value was in both crosses proportionately greater than the corresponding increase in the interval *paba–y*. Secondly, the increase in the recombination frequency in the interval *paba–y* in selection IIb compared with IIa is not associated with a corresponding increase in the interval *an–paba*.

The correlation between recombination in the *ad8* cistron and recombination in the interval *an–paba* has therefore a different basis from the localized negative interference which can be accounted for by discontinuous effective pairing. Presumably such long-range correlations result from heterogeneity in the total frequency of recombination per zygote (Sturtevant, 1955). Similar long-range correlations have been found by Elliott (1959) and have been studied in greater detail by him.

(e) *The frequency of recombination in effectively paired segments*

The data from cross 1 can be used to obtain an estimate of the amount of recombination per unit map length of the genetic material that is effectively paired, in a manner analogous to that used by Chase & Doermann (1958) for bacteriophage. It may be assumed that in individuals in which an exchange occurred in both intervals **c** and **e** both exchanges involved the same effective pairing segment. In this case, all individuals of this type should be effectively paired across interval **d** which lies between **c** and **e**. The proportion of individuals of this type which also have an exchange in **d** will therefore give a measure of the recombination fraction per unit map length of chromosome which is effectively paired.

Since all adenine-independent types were recombinants in **e**, the appropriate fraction is

$$\frac{\mathbf{cd}}{\mathbf{c}+\mathbf{cd}}.$$

The total incidence of recombination in **c** was 0·0338 (Table 5, bottom row). Among recombinants in **d** the incidence of recombination in **c** was 0·0686, but since recombinants in **d** represented only 3·85% of all adenine-independent types, the overall incidence of **cd** recombinants was

$$0{\cdot}0686 \times 0{\cdot}0385 = 0{\cdot}00264;$$

hence

$$\frac{\mathbf{cd}}{\mathbf{c}+\mathbf{cd}} = \frac{0{\cdot}00264}{0{\cdot}0338} = 0{\cdot}0781.$$

A recombination fraction of 0·0781 corresponds to a crossover frequency of 0·0849 (Haldane, 1919).

Since the map length of interval **d** is 0·055 the crossover frequency per unit map length of effectively paired chromosome is

$$\frac{0{\cdot}0849}{0{\cdot}00055} = 154.$$

This admittedly crude calculation implies that the recombination fraction in an

interval that is effectively paired is about 150 times greater than the same fraction in the population as a whole.

(*f*) *The mean length of effectively paired segments*

This can be estimated from the increase in map length in adjacent intervals associated with selection for recombination in a very short interval. For example, in cross 1 the recombination fractions in intervals **b**, **c**, **d**, and **f** in both selection I and selection II (using in this case the weighted and pooled data from selections IIa and IIb—Table 5) were converted crossover fractions (Haldane, 1919). The values in selection II subtracted from those in selection I give the exchange frequency attributable to effective pairing segments encompassing interval **e**. This works out at 41%. Since it has been calculated that the exchange frequency per unit length of effectively paired segments is 154, this increase corresponds to 0·27 as the minimum estimate of the mean length of effectively paired regions. Each effective pairing segment must include all or part of interval **e**. In cross 1 this had an estimated length of 0·25 map unit, but a previous estimate of the length of this interval was nearer 0·1, which is more consistent with other values in the map of the *ad8* cistron. We can therefore make a provisional estimate that the mean length of effective pairing segments is about 0·4 map unit. The mean exchange frequency per segment will therefore be $0{\cdot}004 \times 150$, or 0·6.

(*g*) *The degree of negative interference in different crosses*

If recombination is restricted to effectively paired segments, it may be predicted than an inverse relationship should be found between the length of the interval being used for selection and the degree of negative interference in adjacent intervals, as Calef (1957) and Chase & Doermann (1958) have pointed out. The appropriate data from fourteen crosses, in which the size of the interval used for selection differed by as much as a factor of 300, are listed in Tables 7 and 8. The largest interval available, that between *ad20* and *ad8*, is still much shorter than the estimated mean length of effective pairing segments, and any variation in the degree of negative interference is not therefore expected to be large.

In Table 7, crosses in which the proximal *ad* allele was identical have been listed in order of decreasing recombination fraction between this allele and a second. The last column gives the recombination fraction in the interval between *y* and the proximal *ad* allele among exchanges in the interval between the two *ad* alleles. With two exceptions the data agree with the prediction.

In Table 8 a similar comparison is made with respect to the interval distal to the interval used for selection. In this case the crosses can be compared as a whole since differences in the position of the distal *ad* allele will not alter the length of this interval appreciably. It is clear that in this interval there is no correlation of the type predicted. The reason for this may perhaps be found in the observation that the control recombination fractions in the interval *ad–bi* are heterogeneous. Differences in the degree of negative interference may therefore be obscured by heterogeneity between different crosses.

Table 7. *The relationship between the recombination fraction between different* ad *alleles, and the recombination fraction in the interval between* y *and the proximal* ad *allele*

Cross involving	Recombination fraction ($\times 10^5$)	Recombination fraction between *y* and proximal *ad* allele ($\times 10^2$)
ad20 and *ad8*	160	10·1 ±3·39
ad20 and *ad11*	55	14·3 ±2·02
ad20 and *ad16*	0·5	21·0 ±9·35
ad16 and *ad8*	140	4·32±1·72
ad16 and *ad11*	41	19·3 ±5·28
ad16 and *ad19*	13	24·6 ±2·08
ad19 and *ad8*	120	3·57±3·51
ad19 and *ad11*	15	14·6 ±1·57
ad19 and *ad12*	12	1·67±1·65
ad12 and *ad8*	120	4·32±1·72
ad12 and *ad11*	10	10·0 ±3·87
ad11 and *ad10*	91	9·18±2·92
ad11 and *ad8*	86	2·74±0·85

Table 8. *The relationship between the recombination fraction between two* ad *alleles, and the recombination fraction in the interval between the distal* ad *allele and* bi

Cross involving	Recombination fraction ($\times 10^5$)	Recombination fraction between distal *ad* allele and *bi* ($\times 10^2$)	Control value ($\times 10^2$)*	Difference
ad20 and *ad8*	160	16·4± 4·17	—	10·4 ± 4·17
ad16 and *ad8*	140	22·3± 3·53	—	16·3 ± 3·53
ad12 and *ad8*	120	23·0± 3·57	—	17·0 ± 3·57
ad19 and *ad8*	120	35·7± 9·05	—	29·7 ± 9·05
ad11 and *ad10*	91	35·7± 4·84	6·25±2·02	29·4 ± 6·11
ad11 and *ad8*	86	24·0± 3·19	—	18·0 ± 3·19
ad20 and *ad11*	55	11·3± 1·83	8·04±1·48	3·26± 2·36
ad16 and *ad11*	41	35·7± 6·71	5·80±1·62	29·9 ± 6·90
ad19 and *ad11*	15	21·8± 2·57	3·43±0·77	18·4 ± 2·69
ad16 and *ad19*	13	14·8± 2·28	7·73±1·99	6·07± 3·02
ad19 and *ad12*	12	25·0± 5·59	4·86±1·79	20·1 ± 5·88
ad12 and *ad11*	10	31·7± 3·98	14·2±2·23	17·5 ± 6·38
ad8 and *ad10*	1	39·3± 9·23	—	33·3 ± 9·23
ad20 and *ad16*	0·5	31·6±10·7	7·80±1·82	23·8 ±10·8

* In some crosses a control value could not be obtained and the standard map value of 6·0 units for the interval *y–bi* has been used.

6. DISCUSSION

In the introduction the question was raised whether a process different from recombination of the classical sort was responsible for irregular tetrad ratios, or whether a single mechanism could account for both reciprocal and non-reciprocal

events. There is general agreement (Roman, 1956; Freese, 1957*a*; Case & Giles, 1958) that 3 : 1 ratios in tetrads have a recombinational rather than mutational origin. Since 'gene conversion' is frequently not associated with recombination between markers spanning the locus segregating irregularly, it follows that the frequent simultaneous occurrence of two exchanges, or switches, in a very short chromosome segment must occur and this might be taken as evidence that 'gene conversion' obeyed different rules from crossing over. But the present work suggests that correlated exchanges in very short segments of the genetic material are a property of recombination in general and cannot therefore provide the basis for a distinction between crossing over and 'gene conversion'. The hypothesis that 'gene conversion' is an intra-genic process and crossing over an inter-genic process (Beadle, 1957) is also untenable, as Pontecorvo (1958) has pointed out.

Several authors (e.g. Roman, 1956; Freese, 1957*a*; Chase & Doermann, 1958) have made use of the idea that 3 : 1 ratios in tetrads could be accounted for if recombination occurred by copy-choice provided replication along two mated templates was not necessarily synchronous. Lack of synchrony would permit one template to be vacated by its copy and used to make a second. A specific model of this type was proposed by Freese (1957*a*). He supposed that, within an effectively paired region, switching of the copy from one template to the other occurs repeatedly but is not correlated with a reverse switch, so that within such regions recombination will be generally of the non-reciprocal type. For such a model to have general application the behaviour of all organisms in which tetrad analysis, or half-tetrad analysis, is possible must be taken into account. In yeast in two out of three cistrons analysed (Roman, 1956; Leupold, 1958), recombination between different sites of a cistron appears to be predominantly non-reciprocal. No comparable situation is found in *Aspergillus* (Strickland, 1958*a*, *b*) or *Drosophila*, where non-reciprocal events are very much the exception to the rule. Thus in *Aspergillus* Strickland classified a total of 1245 asci in which all four products of meiosis were recovered, and a further 380 in which three of the four products were recovered. The crosses he used were heterozygous at several loci, and the total number of cases in which any exception from a 2 : 2 ratio would have been detected if it had occurred was 7165 for the complete tetrads and 2119 for the incomplete tetrads, involving a total of 16 loci. Only four exceptional tetrads were found for which orthodox explanations such as contamination and extra mitosis were unlikely. Three of these tetrads involved the same mutant (*bi1*), and in each case a $3bi1^+ : 1bi1$ ratio was found. In the other exceptional ascus, recombination between two very closely linked loci was associated with a 3 : 1 ratio for one of them and in addition a 3 : 1 ratio at a second locus 9 map units distant. A segment of chromosome at least 9 units long was represented three times in the tetrad; it had, apparently, been duplicated twice.

Neurospora appears to occupy an intermediate position with respect to the frequency of tetrads with 3 : 1 ratios (Mitchell, 1955*a*, *b*; Giles *et al.*, 1957; Case & Giles, 1958).

The relative frequencies of reciprocal and non-reciprocal events is clearly

variable from case to case and perhaps from species to species. A model which took account of this variation would be one which postulated a correlation between a switch from one template to a second with a switch in the opposite direction, although the two switches need not necessarily be at precisely the same point, i.e. that recombination is generally a reciprocal process but not necessarily always and precisely so. On such a model a 3 : 1 ratio associated with recombination between spanning markers would require a single event (Fig. 2) instead of three independent events, as on the Freese model. Furthermore, differences in the degree of synchrony of replication would permit variation in the relative frequencies of reciprocal and non-reciprocal events.

A consequence of a unitary model of this type is that in single-strand analysis, among recombinants between two closely linked markers, the frequency of parental combinations with respect to spanning markers should not be much greater than 50%. An excess over 50% is possible if the mean exchange frequency per effective

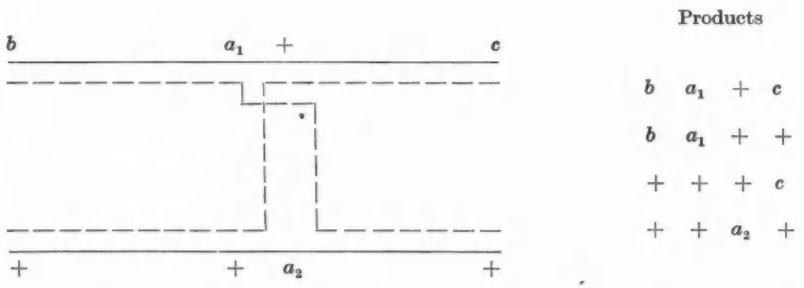


FIG. 2. Proposed origin of a tetrad with a 3 : 1 ratio associated with recombination between spanning markers as a result of a single inexactly reciprocal exchange. Dotted lines represent newly formed products.

pairing segment is not sufficiently great as to randomize the segregation of the spanning markers, since a negative correlation will then occur between exchanges on different sides of the selected exchange due to variability in the position of effective pairing segment (cf. cross 1). Data from the crosses between *pdx* mutants (Mitchell, 1956) and between *pan* mutants (Case & Giles, 1958) of *Neurospora* are in accord with this prediction (Table 9).

These examples are chosen because tetrad analysis in both cases revealed a high frequency of non-reciprocal recombinants. In both cases an excess of parental combinations occurred, but inequality of the two recombinant classes indicated that the frequency of exchange per effective pairing segment was not high. The data of Roman (1956), on the other hand, suggest that 3 : 1 ratios in yeast are not, in the cases studied, associated with recombination. If his results are confirmed they will provide more convincing evidence for more than one mechanism of recombination. At present the evidence is not sufficiently decisive to warrant a definite conclusion.

A further question posed by the experiments discussed in this paper is the significance of localized negative interference. It seems possible that its occurrence may provide an explanation for a number of difficulties encountered in attempting to relate the experimental evidence concerning the mechanism and time of recombination with the cytologically observable sequence of events during nuclear division.

It was an essential feature of postulated mechanisms of recombination involving breakage and reunion of chromosomes that it occurred after zygotene pairing and during or after chromosome duplication (Darlington, 1935). Chromosome duplication was supposed to occur at the end of pachytene. Models of this type have

Table 9. *The relative frequency of different marker combinations among recombinants with respect to closely linked mutants of* Neurospora

Cross	Types selected	Relative frequency of marker combinations (%)					
		Crossovers			Non-crossovers		
		pyr co	+ +	Total	*pyr* +	+ *co*	Total
$\frac{pyr\ pdx\ +}{+\ pdxp\ co}$	Pyridoxin independent	28	8	36	25	39	64
$\frac{pyr\ pdxp\ +}{+\ pdx\ co}$	Pyridoxin independent	8	33	41	31	28	59
		+ +	*ad tryp*		+ *tryp*	*ad* +	
$\frac{+\ +\ B3\ tryp^*}{ad\ B5\ +\ +}$	Pantothenate independent	40	7	47	39	14	53
		ad +	+ *tryp*		+ +	*ad tryp*	
$\frac{ad\ +\ B3\ tryp^*}{+\ B5\ +\ +}$	Pantothenate independent	38	5	43	35	22	57

* *B3* and *B5* determine requirement for pantothenic acid.
References: Mitchell, 1956; Case & Giles, 1958.

been repeatedly questioned for reasons summarized and added to recently by Pontecorvo (1958). A model in which recombination is the result of copy-choice at the time of replication is now generally preferred, but in its simplest form such a model has its own difficulties. Recombination by copy-choice requires homologous contact between chromosomes at the time of replication, but replication of DNA had been shown to occur well before zygotene pairing in a number of organisms (see Taylor, 1957).

Taylor (1957) concluded that any hypothesis requiring chromosome duplication after zygotene pairing was untenable. This led him to suggest a branched structure for chromosomes and two mechanisms of recombination, one occurring at the time of replication by copy-choice and involving the side chains, and one occurring after replication by breakage and reunion and involving the chromosome backbone.

But the evidence against a branched linkage structure is convincing and Taylor's model is therefore unsatisfactory.

If recombination is the result of copy-choice it becomes necessary to conclude that it occurs before, and independently of, zygotene pairing as Pontecorvo (1958) has suggested.

The discovery of the extreme discontinuity in the distribution of recombination events in *Aspergillus* (Pritchard, 1955) suggested how this apparent paradox might be resolved. It was pointed out then that effective pairing, a condition for recombination, was not necessarily identical with pairing observed cytologically. A provisional estimate now indicates that the frequency of exchange per effective pairing segment is about 0·6, corresponding to a recombination fraction of about 35%. This means that the frequency of recombination between loosely linked markers is determined principally by the frequency of effective pairing between them, i.e. that effective pairing is a limiting factor in recombination.

Thus homologous chromosomes need necessarily be in contact over only a small fraction of their total length to account for the recombination fractions observed. Such chromosomes need not be paired in the cytologically visible sense. All that would be necessary would be occasional points, or very short segments, of contact between them. Chiasmata would be produced at the time of replication (before cytologically visible pairing) but observed only at diplotene.

It is assumed in this argument that effective pairing is synonymous with homologous contact. Although this idea is attractive it is clear that discontinuous contact is not the only source of discontinuity along chromosomes that could lead to clustering of recombination events. There is nevertheless evidence which at first sight seems to support this view. It is well known that the frequency of chiasmata in triploids and trisomics is greater than in the corresponding diploid (e.g. Darlington, 1934, 1941; Upcott, 1935), and there is evidence for a corresponding increase in the recombination frequency in individuals of this type in *Drosophila*, although the effect of recombination on disjunction in triploids complicates the interpretation of these results (see Schultz & Redfield, 1951). A similar situation seems to exist in trisomic *Aspergillus* (Pritchard & Siddiqi, unpublished).

If contact between homologues were not a limiting factor in recombination these observations could only be interpreted in terms of an unspecified effect of the presence of a third homologue. If homologous contact were limiting, on the other hand, an increase would be predicted simply as a result of the increased probability of contact between two homologous points when three are present. This was pointed out by Darlington (1941) for the case of an organism in which cytologically visible pairing was incomplete. The present hypothesis is that homologous contact is always incomplete—more precisely, discontinuously so—irrespective of the pattern of cytologically visible pairing.

It is not, of course, suggested that all observations of this sort can be accounted for entirely in this way. Sturtevant's (1951) observation of an increase in recombination in chromosome IV of *Drosophila melanogaster* in diplo-IV triploids is a case in point.

It is assumed, then, that the probability of recombination between loosely linked loci has three components: the frequency of effective pairing, the length of effectively paired segments, and the frequency of exchange per unit length of such segments. If this is correct, it suggests how large differences in the frequency of recombination per unit of physical length of the genetic material in different organisms could arise, although the underlying mechanism of recombination was the same in all of them. A comparison between *Aspergillus* and bacteriophage will make this clear.

We can use the cistron as an approximate standard of physical length in view of its presumed direct or indirect role as a template for synthesis of a polypeptide, and Pontecorvo & Roper's (1956) estimate that in organisms as different as *Drosophila* and phage the amount of DNA per cistron is probably similar. The genetic length of the *ad8* cistron is about 0·2 map unit. This value seems typical for *Aspergillus* (e.g. Roper, 1950). The genetic length of the rIIA and rIIB cistrons in phage T4, on the other hand, is about 3 and 5 units respectively (Chase & Doermann, 1958), which also seems to be typical (e.g. Streisinger & Franklin, 1956). We can calculate what these values would correspond to if effective pairing were complete in the two organisms. The *ad8* cistron would become 30 ($0{\cdot}2 \times 150$) units long, and the rIIA and rIIB cistrons 30 (3×10) and 50 (5×10) units respectively. A conversion factor of 10 is used for phage, since Chase & Doermann found that if only those individuals in which a given interval was effectively paired were considered the recombination fraction was 6–14 times that in the population as a whole. Thus the 20-fold difference in the probability of recombination per unit physical length of the genetic material in the two organisms may turn out to be due largely to differences in the frequency or length of effective pairing segments.

In comparison with these values the high frequency of double exchanges required for the incorporation of very small transducing or transforming segments of DNA by copy-choice no longer seems remarkable. Indeed, there is at present no compelling evidence against the view that fundamentally the same mechanism is involved in recombinational processes apparently as different as transformation and crossing over.

SUMMARY

In the analysis of recombination in *Aspergillus nidulans* coincidence values of about 1 are found in 3-point tests using markers more than a few map units apart. In comparable tests in which the marked intervals were very short (0·1 map unit or less), coincidence values of over 100 had been found. To account for this difference it was proposed that a necessary condition for recombination, termed 'effective pairing', was realized at any particular point on the chromosome in only a small fraction of a population of cells at meiosis. It was supposed that when effective pairing occurred it extended over a very short segment of the chromosome and that the probability of recombination in the effectively paired segment was high, i.e. about 1. Coincidence values greater than unity would be

a necessary consequence of such a situation provided the intervals in question had a total length not much greater than that of effective pairing segments.

The experiments described in this paper were undertaken in an attempt to measure the mean length of effectively paired segments, their distribution, and the frequency of exchange within them. The data suggest a mean length of about 0·4 map unit, a mean exchange frequency of about 0·6, and a distribution which is variable, perhaps random.

The occurrence of localized negative interference suggests a way in which a number of difficulties encountered in relating the experimental evidence concerning the time and mechanism of recombination with the cytological evidence concerning the sequence of events at meiosis might be resolved. The data indicate that the frequency of recombination between linked loci is a measure principally of the frequency of effective pairing between them. If effective pairing is synonymous with homologous contact between chromosomes, and evidence is presented which suggests this may be the case, it becomes possible to construct a simple model which is compatible with the view that recombination takes place before chromosomes are paired, in the cytologically observable sense (i.e. before zygotene), at meiosis.

The recombination events occurring within effectively paired regions are generally, although possibly not exclusively, reciprocal. Non-reciprocal recombinants have been encountered in *Aspergillus* and other organisms, characterized by the occurrence of 3 : 1 ratios in tetrads. On the basis of evidence currently available it does not seem necessary to invoke a special mechanism of recombination, distinct from crossing over, to account for the formation of non-reciprocal recombinants. A single mechanism of recombination of the copy-choice type which, although primarily a reciprocal process, is nevertheless not necessarily exactly so or always so in detail, will account for the observed results.

The author is especially indebted to Prof. G. Pontecorvo, F.R.S., and Dr. J. A. Roper for much advice and criticism during the course of this work and in preparation of the manuscript.

REFERENCES

BEADLE, G. W. (1957). The role of the nucleus in heredity. *The Chemical Basis of Heredity*. Baltimore: Johns Hopkins Press. Pp. 3–22.

CALEF, E. (1957). Effect on linkage maps of selection of crossovers between closely linked markers. *Heredity*, **11**, 265–279.

CASE, M. E. & GILES, N. H. (1958). Evidence from tetrad analysis for both normal and abnormal recombination between allelic mutants in *Neurospora crassa*. *Proc. Nat. Acad. Sci., Wash.*, **44**, 378–390.

CAVALLI-SFORZA, L. L. & JINKS, J. L. (1956). Studies on the genetic system of *Escherichia coli K-12*. *J. Genet.* **54**, 87–112.

CHASE, M. & DOERMANN, A. H. (1958). High negative interference over short segments of the genetic structure of bacteriophage T4. *Genetics*, **43**, 332–353.

CHOVNICK, A. (1958). Aberrant segregation and pseudoallelism at the garnet locus in *Drosophila melanogaster*. *Proc. Nat. Acad. Sci., Wash.*, **44**, 333–337.

DARLINGTON, C. D. (1934). The origin and behaviour of chiasmata. VII. *Zea Mays*. *Z. indukt. Abstamm.- u. VererbLehre*, **67**, 96–114.

DARLINGTON, C. D. (1935). The time, place and action of crossing-over. *J. Genet.* **31,** 185–212.
DARLINGTON, C. D. (1941). The causal sequence of meiosis. II. Contact points and crossing-over potential in a triploid *Fritillaria*. *J. Genet.* **41,** 35–48.
DEMEREC, M. (1928). Mutable characters of *Drosophila virilis*. I. Reddish-alpha body character. *Genetics*, **13,** 359–388.
DE SERRES, F. J. (1958). Recombination and interference in the *ad-3* region of *Neurospora crassa*. *Cold Spr. Harb. Symp. quant. Biol.* **23,** 111–118.
ELLIOTT, C. G. (1959). In the press.
EMERSON, S. (1956). Notes on the identification of different causes of aberrant tetrad ratios in *Saccharomyces*. *C. R. Lab. Carlsberg*, **26,** 71–86.
FREESE, E. (1957 *a*). The correlation effect for a histidine locus of *Neurospora crassa*. *Genetics*, **42,** 671–684.
FREESE, E. (1957 *b*). Über die Feinstruktur des genoms im Bereich eines *pab* Locus von *Neurospora crassa*. *Z. indukt. Abstamm.- u. VererbLehre*, **88,** 388–406.
GILES, N. H. (1951). Studies on the mechanism of reversion in biochemical mutants of *Neurospora crassa*. *Cold Spr. Harb. Symp. quant. Biol.* **16,** 283–313.
GILES, N. H. (1955). Forward and backward mutation at specific loci in *Neurospora*. *Brookhaven Symp. in Biol.*, **8,** 103–125.
GILES, N. H., DE SERRES, F. J., & BARBOUR, E. (1957). Studies with purple adenine mutants of *Neurospora crassa*. II. Tetrad analysis from a cross of an *ad-3A* mutant with an *ad-3B* mutant. *Genetics*, **42,** 608–617.
GLASS, B. (1957). *A summary of the Symposium on The Chemical Basis of Heredity*. Baltimore: Johns Hopkins Press. Pp. 257–834.
GREEN, M. M. (1954). Pseudo-allelism at the vermilion locus in *Drosophila melanogaster*. *Proc. Nat. Acad. Sci., Wash.*, **40,** 92–99.
GREEN, M. M. & GREEN, K. C. (1949). Crossing over between alleles at the lozenge locus in *Drosophila melanogaster*. *Proc. Nat. Acad. Sci., Wash.*, **35,** 586–591.
HALDANE, J. B. S. (1919). The combination of linkage values, and the calculation of distances between loci of linked factors. *J. Genet.*, **8,** 299–309.
HEXTER, W. M. (1958). On the nature of the garnet locus in *Drosophila melanogaster*. *Proc. Nat. Acad. Sci., Wash.*, **44,** 768–771.
KÄFER, E. (1958). An 8-chromosome map of *Aspergillus nidulans*. *Advanc. Genet.* **9,** 105–145.
LEDERBERG, J. (1955). Recombination mechanisms in bacteria. *J. cell. comp. Physiol.* **45** (Suppl. 2), 75–107.
LEUPOLD, U. (1958). Studies on recombination in *Schizosaccharomyces pombe*. *Cold Spr. Harb. Symp. quant. Biol.* **23,** 161–170.
LEWIS, E. B. (1945). The relation of repeats to position effect in *Drosophila melanogaster*. *Genetics*, **30,** 137–166.
LEWIS, E. B. (1952). The pseudoallelism of white and apricot in *Drosophila melanogaster*. *Proc. Nat. Acad. Sci., Wash.*, **38,** 953–961.
LINDEGREN, C. C. (1955). Non-Mendelian segregation in a single tetrad of *Saccharomyces* ascribed to gene conversion. *Science*, **121,** 605–607.
MACKENDRICK, M. E. & PONTECORVO, G. (1952). Crossing over between alleles at the *w* locus in *Drosophila melanogaster*. *Experientia*, **8,** 390.
MITCHELL, H. K. (1957). Crossing over and gene conversion in *Neurospora*. *The Chemical Basis of Heredity*. Baltimore: Johns Hopkins Press. Pp. 94–113.
MITCHELL, M. B. (1955 *a*). Aberrant recombination of pyridoxin mutants of *Neurospora*. *Proc. Nat. Acad. Sci., Wash.*, **41,** 215–220.
MITCHELL, M. B. (1955 *b*). Further evidence of aberrant recombination in *Neurospora*. *Proc. Nat. Acad. Sci., Wash.*, **41,** 935–937.
MITCHELL, M. B. (1956). A consideration of aberrant recombination in *Neurospora*. *C. R. Lab. Carlsberg*, **26,** 285–298.
PONTECORVO, G. (1958). *Trends in Genetic Analysis*. New York: Columbia University Press.
PONTECORVO, G. & KÄFER, E. (1958). Genetic analysis based on mitotic recombination. *Advanc. Genet.* **9,** 71–104.
PONTECORVO, G. & ROPER, J. A. (1956). Resolving power of genetic analysis. *Nature, Lond.*, **178,** 83–84.

PONTECORVO, G., ROPER, J. A., HEMMONS, L. M., MACDONALD, K. D. & BUFTON, A. W. J. (1953). The genetics of *Aspergillus nidulans*. *Advanc. Genet.* **5**, 141–238.

PRITCHARD, R. H. (1955). The linear arrangement of a series of alleles in *Aspergillus nidulans*. *Heredity*, **9**, 343–371.

ROMAN, H. (1956). Studies of gene mutation in *Saccharomyces*. *Cold Spr. Harb. Symp. quant. Biol.* **21**, 175–185.

ROPER, J. A. (1950). A search for linkage between genes determining vitamin requirements. *Nature, Lond.*, **166**, 956.

ROPER, J. A. & PRITCHARD, R. H. (1955). The recovery of the reciprocal products of mitotic crossing over. *Nature, Lond,*. **175**, 639.

ROTHFELS, K. H. (1952). Gene linearity and negative interference in crosses of *Escherichia coli*. Genetics, **37**, 297–311.

SCHULTZ, J. & REDFIELD, H. (1951). Interchromosomal effect on crossing over in *Drosophila*. *Cold Spr. Harb. Symp. quant. Biol.* **16**, 175–197.

SPURWAY, H. & PHILIP, U. (1952). Genetics and cytology of *Drosophila subobscura*. VII. Abnormal gene arrangements in element A. *J. Genet.* **51**, 198–215.

ST. LAWRENCE, P. (1956). The *q* locus of *Neurospora crassa*. *Proc. Nat. Acad. Sci., Wash.*, **42**, 189–194.

STREISINGER, G. & FRANKLIN, N. C. (1956). Mutation and recombination at the host range genetic region of phage T2. *Cold Spr. Harb. Symp. quant. Biol.*, **21**, 103–111.

STRICKLAND, W. N. (1958 *a*). Abnormal tetrads in *Aspergillus nidulans*. *Proc. roy. Soc.* B, **148**, 533–542.

STRICKLAND, W. N. (1958 *b*). An analysis of interference in *Aspergillus nidulans*. *Proc. roy. Soc.* B, **149**, 82–101.

STURTEVANT, A. H. (1951). A map of the fourth chromosome of *Drosophila melanogaster*, based on crossing over in triploid females. *Proc. Nat. Acad. Sci., Wash.*, **37**, 405–407.

STURTEVANT, A. H. (1955). Evaluation of recombination theory. *J. cell. comp. Physiol.* **45** (Suppl. 2), 237–242.

TAYLOR, J. H. (1957). The time and mode of duplication of chromosomes. *Amer. Nat.* **91**, 209–221.

UPCOTT, M. (1935). The cytology of triploid and tetraploid *Lycopersicum esculentum*. *J. Genet.* **31**, 1–19.

WEINSTEIN, A. (1918). Coincidence of crossing over in *Drosophila melanogaster* (*ampelophila*). *Genetics*, **3**, 135–172.

Genet. Res., Camb. (1960), **1**, *pp.* 25–38
With 3 text figures
Printed in Great Britain

'Heterosis' in F_1 mice in a cold environment

BY S. A. BARNETT AND ELIZABETH M. COLEMAN
Department of Zoology, The University, Glasgow

(*Received* 25 *April* 1959)

1. INTRODUCTION

The terms 'hybrid vigour' and 'heterosis' refer to the fact that outbred organisms are often more successful, on some measurable criterion, than inbred individuals of the same species. The criteria usually employed are measures of fertility or growth, but resistance to disease and yet others may be used. A current explanation of heterosis is in terms of the effects on ontogeny of gene interaction. It is assumed that there is an optimal direction and rate for each developmental process, at least for a given environment, and that the optimum course is followed more closely by heterozygous than by homozygous individuals (Waddington, 1942; Haldane, 1949, 1954; Lerner, 1954).

If heterosis is due to a 'buffering' action of the genes in ontogeny, it should follow that it would be more evident in an environment which, while permitting survival and reproduction, departs substantially from the optimum: in such an environment the effect of heterozygous loci ought to be magnified. This has been tested, in the work described below, on inbred and F_1 mice reared in two environments. It had already been found that mice of strains A, A2G and C57BL could be maintained as permanent breeding stocks in an environment kept at $-3°$ C. (Barnett & Manly, 1954, 1959). Fertility is lower in these conditions than at 21° C., especially in strain C57BL. The account that follows shows that the F_1 mice obtained by crossing A2G and C57BL mice are more fertile than either parent strain at each temperature, but that the difference is much greater at $-3°$ C.; and F_1 variation in certain parameters is lower at both temperatures but especially at $-3°$ C.

2. METHOD

The parental mice were of the A2G and C57BL strains, rigorously inbred. A breeding stock of each strain is maintained in each of two constant-temperature rooms, kept respectively at about 21° C and about $-3°$ C. They are fed on diet 41, and have cotton wool for nesting. The breeding performance of these mice, and the conditions in which they are kept, have been fully described elsewhere (Barnett, 1956; Barnett & Manly, 1956, 1959).

To provide the F_1 mice in the cold room, fourteen pairs were mated; in each, the female was of the C57BL strain and the male A2G. The age at mating was about 6 weeks. Only five pairs produced young within 8 weeks of mating. In three of these pairs the male was of the fifth generation in the cold and the female of the

Table 1. *Breeding performance*

	21° C.			−3° C.		
	A2G	C57BL	F_1	A2G	C57BL	F_1
No. of pairs	15	15	11	19	10	12
Litters born per pair	5·1 ± 0·56	5·1 ± 0·27	4·6 ± 0·23	2·8 ± 0·20	2·4 ± 0·58	3·1 ± 0·08
Litters weaned per pair	4·5 ± 0·52	3·7 ± 0·40	4·5 ± 0·27	1·3 ± 0·19	1·1 ± 0·55	3·0 ± 0·12
Young born per pair	27·1 ± 3·06	33·5 ± 2·85	43·8 ± 0·99	10·9 ± 1·09	12·3 ± 3·16	27·7 ± 1·09
Young weaned per pair	20·7 ± 2·59	20·4 ± 3·46	39·1 ± 1·82	4·3 ± 0·73	5·3 ± 1·59	22·7 ± 1·51
No. of young born per litter	5·3 ± 0·20	6·5 ± 0·28	9·5 ± 0·40	4·1 ± 0·24	5·1 ± 0·48	9·0 ± 0·71
No. of young weaned per weaned litter	4·7 ± 0·22	5·6 ± 0·30	8·8 ± 0·37	3·2 ± 0·28	4·8 ± 0·59	7·6 ± 0·30

The standard error is given with each mean.

first generation: that is, the female's parents had been transferred to the cold before mating. In the other two pairs the male was of the eighth generation in the cold, the female of the second. In the warm room, the corresponding group consisted of five similar pairs, all of which produced young. The F_1 young of these pairs, in both temperatures, were weaned at 3 weeks. Some were mated, each to a member of the same litter, at 5 weeks; and their breeding performance was recorded until they reached 28 weeks. The young of these pairs were weighed to the nearest 0·5 g. at the age of 3 weeks.

Others of the F_1 young in the warm room were 'stressed' by transfer to the cold room at 22 days. Each mouse was placed alone in a cage containing cotton wool. As a control a litter-mate of the same sex was put in the same conditions in the warm room. Mortality and growth in these mice were recorded to the age of 6 weeks, when all survivors were killed. Comparison was made with mice of the parent strains similarly treated. The effect of cold stress on young inbred mice has been more fully described by Barnett, Coleman & Manly (1960).

3. RESULTS

(*a*) *Breeding performance*

The data on the breeding of the three kinds of pairs in each temperature are given in Table 1. The performance of the two parent strains has already been discussed by Barnett & Manly (1959).

At 21° C., as expected, the F_1 pairs did better than either parent strain. The superiority of the F_1 pairs appeared in the number of young per litter, both at birth and at weaning. There were consequently more F_1 young, both born and weaned, per pair than C57BL or A2G. The superiority of the F_1 strains was especially marked at weaning, since their losses between birth and 3 weeks were

Table 2. *Percentage loss of young between birth and weaning*

	21° C.	−3° C.
A2G	23·6	40·6
C57BL	39·1	56·9
F_1	10·7	18·1

See Table 1 for numbers of pairs and of young.

much lower than were those of the parent strains (Table 2): the number of young weaned per pair was 1·9 times the mean of the inbred strains.

At −3° C. the breeding of the F_1 mice was again better than that of the parent strains at the same temperature, but the difference was much greater than that in the warm. This was shown in the number of litters born, and still more in the number weaned. Among the inbred mice losses of whole litters were common at −3° C. (Barnett & Manly, 1959), but out of the thirty-seven F_1 litters only one

such loss occurred. Litter size, both at birth and at weaning, was about twice that of the parent strains. Consequently, the number of young born per pair was more than twice that in the inbred stocks, and the number weaned per pair was 4·7 times the mean of the parent strains.

The number of young weaned per pair is, of all the parameters measured, the most significant both from a biological point of view (that is, for fitness) and from the standpoint of a stockbreeder. Mortality after 3 weeks was negligible; consequently each mouse reared to that age was a potential breeder or, if kept but not mated, had a very high probability of becoming an adult. From this point of view there was, as would be expected, a marked advantage in hybridity at the (near optimum) temperature of 21° C., but a much greater advantage at −3° C.

The preceding paragraph requires qualification. Not all the mice that reached 3 weeks were fecund. Barnett & Manly (1959) had three barren C57BL pairs out of ten at −3° C., and other pairs have proved to be barren in subsequent breeding (unpublished). There was a smaller incidence of infecundity among the A2G mice. But, of the eleven F_1 pairs at −3° C., all were fertile and (as further discussed below) the degree of fertility varied little among the pairs. It follows that, of the young weaned by inbred pairs at −3° C., a substantial proportion would be unable to contribute to the next generation; while, of the young weaned by F_1 pairs, all (or nearly all) could be expected to be able to do so. The available data do not allow an exact estimate of the number of fertile young produced per pair, but it can be concluded that the figure for F_1 pairs in the cold would be more than 4·7 times the mean of the parent strains.

A further qualification concerns the interpretation of the figures for number of litters and of young born and weaned per pair (but not per litter). In these experiments breeding was recorded up to a given age. Consequently, the age at which the breeding began was a factor in reproductive performance. Table 3 shows the mean

Table 3. *Age in days of females at birth of first litter*

		A2G	C57BL	F_1
21° C.	Range	61–99	59–131	52–66
	Mean with standard error	73·6 ± 3·09	79·4 ± 4·80	59·0 ± 1·15
−3° C.	Range	79–210	97–183	62–82
	Mean with standard error	117·1 ± 9·85	118·0 ± 10·39	72·4 ± 1·92

The numbers of females are the same as the numbers of pairs in Table 1.

age at first litter for each class of female. In both temperatures the F_1 mice began earlier than the inbred mice at the same temperature; further, their range was much smaller and (as discussed further in §3(*d*) below) variation was lower. Thus

the superior performance of the F_1 mice, especially in the cold, was partly a consequence of their earlier maturity. Delay in the attainment of maturity, shown by Barnett & Coleman (1960) to occur in A2G females in the cold, was evidently not so great in the F_1 mice.

Table 4. *Mass of young weaned per pair; numbers of pairs, and means with standard errors*

		21° C.	−3° C.
A2G	No. of pairs	12	13
	Mass weaned per pair (g.)	240·9 ± 39·08	44·4 ± 12·41
C57BL	No. of pairs	14	5
	Mass weaned per pair (g.)	189·2 ± 25·66	56·3 ± 23·42
F_1	No. of pairs	11	12
	Mass weaned per pair (g.)	350·5 ± 5·28	193·4 ± 12·05

The number of pairs in some classes is smaller than that in Table 1, since in some instances not all the young were weighed at 3 weeks.

Table 5. *Mean weights, with standard errors, of young aged 3 weeks, of inbred and F_1 pairs*

		21° C.		−3° C.	
		♂	♀	♂	♀
C57BL	No.	88	96	18	19
	g.	8·4 ± 0·15	8·2 ± 0·14	7·4 ± 0·27	7·1 ± 0·23
A2G	No.	107	118	55	56
	g.	10·2 ± 0·15	9·8 ± 0·13	8·5 ± 0·17	8·3 ± 0·16
F_1	No.	225	242	122	151
	g.	9·2 ± 0·09	8·7 ± 0·08	8·5 ± 0·08	8·5 ± 0·07

(*b*) *Body weights of young at three weeks*

The preceding account has expressed the performance of breeding pairs in terms of the *numbers* of young born and weaned. A complete picture of the results of breeding must also include a statement of the *mass* of the young produced. Table 4 shows that at 21° C., in terms of total mass of 3-week young per pair, the F_1 mice

do about 1·6 times as well as the mean of the parent strains. At −3° C. the corresponding figure is 3·8. From this point of view the advantage of hybridity is twice as great at −3° C. as at 21° C.

These figures, taken by themselves, could be a result of the production of many small mice or fewer large ones. The fact that the litters of F_1 pairs, both at birth and at weaning, were much larger than those of the parent strains suggests the

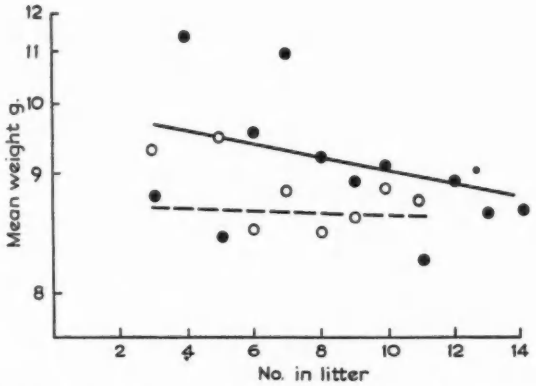


Fig. 1. The relationship between number in litter at 3 weeks and mean body weight (on a logarithmic scale) at that age: F_2 males. The fitted regression lines are shown. Solid circles: 21° C.; open circles: −3° C.

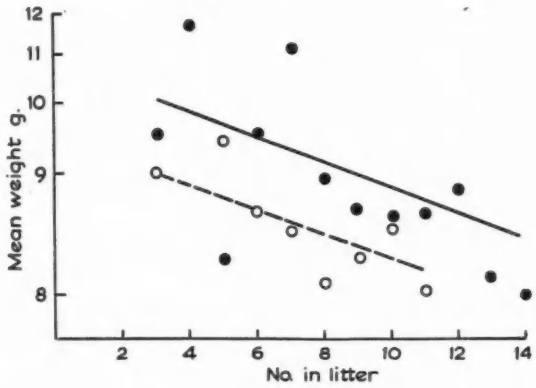


Fig. 2. As Fig. 1: F_2 females.

possibility that the weights of the individual mice would be lower than those from the smaller inbred litters. (For references to the literature on the relationship of body weight to number in litter, see Barnett & Manly, 1959.) Table 5 shows that, despite the much larger litters of the F_1 pairs, the mean weights at weaning of the young at 21° C. lay between those of the parent strains. At −3° C. they were

almost identical with those of the A2G mice, and significantly higher than those of the C57BL mice.

Figs. 1 and 2 show the relation of log weight at 3 weeks to number in litter at that age. Especially for the females at both temperatures there is a clear indication of the expected decline in weight with increased number in litter (cf. Ashoub, Biggers, McLaren & Michie (1958), for a similar sex difference in TO mice). Nevertheless, even at −3° C., the figures show that members of litters of nine, ten and eleven still averaged above 8 g. at 3 weeks. The corresponding data for strains A2G and C57BL have been given by Barnett & Manly (1959), but with the sexes not separated. They found an indication of decline in body weight with litter size in strain C57BL at both temperatures, but in strain A2G only at −3° C.

In general, then, while the number of hybrid mice produced is much higher than that of inbred mice, the body weights are nevertheless maintained at a level similar to that of the parent strains.

(c) *Resistance of young to cold*

Table 6 shows the mortality which resulted from transferring A2G and F_1 mice at 22 days, and C57BL mice at 35 days, from 21° C. to −3° C. (cf. Barnett &

Table 6. *Mice aged 3 weeks, reared at 21° C.: mortality during 7 days' exposure alone in a cage*

		Exposed at −3° C.		Controls at 21° C.	
		♂	♀	♂	♀
A2G	No.	20	21	15	15
	% died	35·0	42·9	0·0	0·0
C57BL	No.	15*	14*	8	8
	% died	46·7	50·0	0·0	0·0
F_1	No.	24	24	20	24
	% died	12·5	8·3	0·0	4·2

* Aged 5 weeks at transfer to −3° C.

Manly, 1958; Barnett, Coleman & Manly, 1960). The hybrid young showed themselves more resistant to cold even than the A2G mice.

Fig. 3 (upper graphs) compares the growth of hybrid mice 'stressed' at 22 days with that of A2G mice similarly treated. There are no comparable figures for C57BL mice, since these were stressed only at the age of 5 weeks. Fig. 3 (lower graphs) gives similar data for the controls, including those of strain C57BL. Of the two parent strains the A2G mice were the heavier at ordinary temperatures.

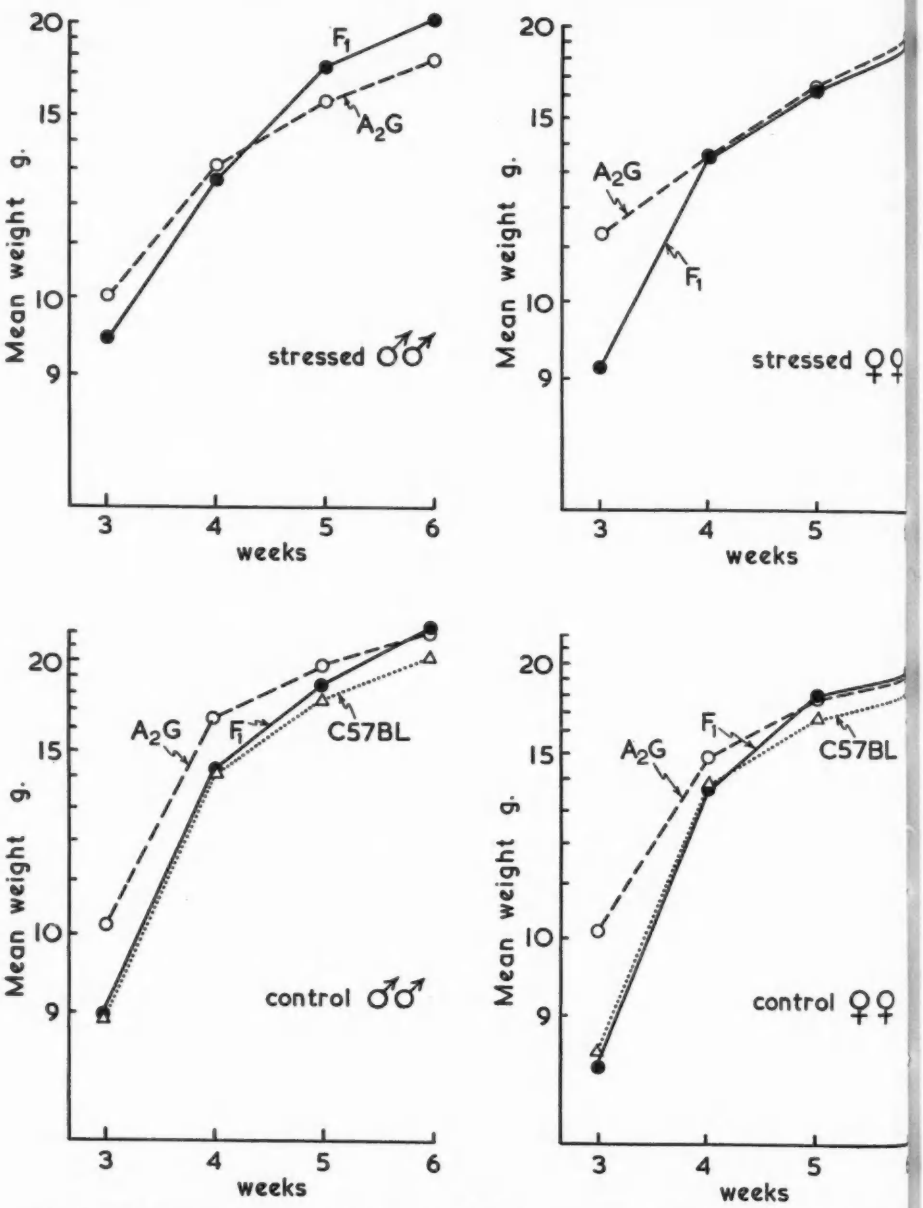


Fig. 3. Above: growth of F_1 and A2G mice after transfer to −3° C. at the age of 22 days. Below: growth of F_1, A2G and C57BL mice at 21° C. Males on left, females on right. The ordinates give weight on a logarithmic scale.

The stressed A2G mice of which the weights were recorded formed a biased sample, since they inevitably came from the two-thirds that survived the stress. By contrast, the F_1 mice, with their lower mortality, were more nearly a representative sample. In each sex, and in each temperature, the F_1 mice had a higher growth-rate. Since the mean weight of the F_1 mice at 3 weeks was lower than that of this sample of A2G mice, the growth curves in each instance converge. By 6 weeks the stressed male F_1 mice were heavier than the A2G males. In other classes the weights at 6 weeks of the A2G and F_1 mice were almost identical. The control mice of the C57BL strain were lighter than the corresponding F_1 mice at 6 weeks.

The superior performance of young F_1 mice on being transferred to the cold is relevant to the greater fertility of the F_1 mice bred in the cold. It has been shown above that the greater number of young they rear to weaning is partly a result of a much lower post-natal mortality. A high degree of resistance to cold is thus reflected in the observations on mortality both of F_2 nestlings born in the cold and of stressed F_1 mice.

(*d*) *Variation*

The preceding account has dealt with *average* performance in reproduction and resistance to cold. There remains the question of the extent to which *variation* in performance is influenced by hybridity (cf. Grüneberg, 1954; McLaren & Michie, 1954).

Table 7 gives the coefficients of variation for the parameters of reproduction, for each class of mice. As already reported by Barnett & Manly (1959), the mice

Table 7. *Coefficients of variation, per cent*

	A2G		C57BL		F_1	
	21° C.	−3° C.	21° C.	−3° C.	21° C.	−3° C.
Litters born per pair	41·0 (29·8)	29·7 (21·2)	19·7	64·1 (26·3)	16·6*	9·0*†
Litters weaned per pair	39·5 (35·5)	59·4 (39·0)	40·8	72·5 (46·4)	20·0	13·5‡
Young born per pair	42·3 (31·9)	42·4 (35·1)	31·9	77·1 (34·1)	7·5*§	13·6‡
Young weaned per pair	46·8 (41·2)	72·6 (53·4)	63·6	90·0 (51·8)	15·5	23·0
Young born per litter	33·1	40·5	37·6	46·1	30·0	48·2
Young weaned per weaned litter	38·0	41·7	39·4	35·2	29·3	23·4

The figures in brackets are the coefficients calculated only for those pairs whose record was above zero.

* Variance significantly different from A2G ($P < 0{\cdot}05$)
† " " " " C57BL ($P < 0{\cdot}01$)
‡ " " " " " ($P < 0{\cdot}1$)
§ " " " " " ($P < 0{\cdot}05$)

In each instance comparison was made between F_1 and inbred mice in the same temperature.

of the inbred strains tend to vary more at $-3°$ C. than at $21°$ C. The effect of hybridity was to reduce variation in most of the parameters of reproduction, at both temperatures. The F_1 mice showed greater uniformity in the number of litters born per pair, even though their average performance in this respect differed little from that of the parent strains. In litters weaned per pair the effect of environmental temperature on variation was very marked: the low temperature did not increase variation in the F_1 mice, but greatly increased it in both parent strains if pairs which weaned no young are included. In number of young born and number of young weaned per pair, the F_1 mice showed greater uniformity at both temperatures. There was no significant effect of temperature on variation in numbers of young born or weaned *per litter*.

4. DISCUSSION

Heterosis may be defined as the occurrence of greater fitness in outbred than in inbred individuals of the same species. 'Fitness' is used in a Darwinian sense; its meaning has been fully discussed by Thoday (1953). As indices of fitness it is reasonable to use such parameters as the number of young weaned per pair or resistance to disease, but not size or growth rate: there is no *a priori* likelihood that more rapid growth or greater adult body weight will be advantageous. The fact that outbreeding does sometimes lead to increased growth has led Dobzhansky (1950) to propose 'luxuriance' as a name for this phenomenon, in order to distinguish it from heterosis as defined above.

'Inbreeding depression' in domestic mammals in respect of fertility and of viability of the young is well known (Donald, 1955). This is the inverse of heterosis. Gates (1925) and Chai (1956) have recorded examples of higher fertility in outbred mice. Marshak (1936) and Falconer & King (1949) have also observed luxuriance, or 'weight heterosis', in F_1 mice. Butler (1958) found that F_1 mice from strains BALB and C57BL had larger litters than the parent strains. The data from our control mice illustrate the superior breeding performance of F_1 mice in near-optimum conditions. They also give some evidence on the extent to which different stages in the production of young were influenced by hybridity. The number of *litters* born to F_1 pairs in the warm room was not increased; nor was the number of litters weaned by them significantly different from that of the inbred pairs. But the numbers of *young* born and weaned were greatly increased. Three groups of factors were no doubt involved: (i) conditions in the uterus; (ii) conditions in the nest, including milk supply; (iii) the genetical character of the F_2 young. (i) and (ii) have been discussed by Barnett & Manly (1959) in relation to reproduction in the inbred stocks. The data on the F_1 mice do not allow us to distinguish with certainty the effects of these influences from those of (iii). However, the facts that (*a*) the number weaned per litter by the F_1 mice was nearly twice that of inbred strains and (*b*) the F_2 mice at weaning were intermediate in weight between the parent strains show that the F_1 females must have had a higher milk yield than the inbred females.

The main interest of our breeding data is in the effect of hybridity in the cold environment. Here the superiority of the F_1 mice was far more marked. The number of young born per pair was more than twice the mean of the inbred strains, and presumably reflects a better uterine environment. Still more impressive was the nearly fourfold increase in the number of young weaned per pair. As in the warm environment, this presumably represented the combined effects of maternal efficiency and the 'vigour' of the F_2 young. The superior vigour, in the sense of resistance to cold, of young F_1 mice reared in the warm was clearly shown in the results of subjecting them to cold stress at weaning.

An important question is to what extent the performance of the F_1 mice in the cold environment was more uniform, as well as more successful, than that of the parent strains. Table 7 shows that percentage coefficients of variation for certain aspects of reproductive performance in the F_1 mice were much lower than in the inbred mice. This held especially for the numbers of young born and weaned per pair in both temperatures. There was also a marked regularity in number of litters born per pair in the F_1 mice at $-3°$ C.

The coefficients of variation for the F_1 mice were low in both temperatures. Except in the number of young *born* per *litter*, the pattern of increased variation in the cold, which was especially shown by the C57BL mice, did not appear in the hybrids. In the F_1 mice the coefficients for numbers of young born and weaned per pair were higher in the cold than in the warm, but variation in these parameters was in any case very low in both environments.

The comments above apply to comparisons of variation estimated for all the pairs in each class. However, some inbred pairs were barren, namely, one A2G pair in each temperature and three C57BL pairs at $-3°$ C.; further, one fecund A2G pair at $-3°$ C. reared no young to 3 weeks (Barnett & Manly, 1959). In Table 7 the figures in brackets give the coefficients of variation calculated with these 'zeros' omitted; they show that, especially in the C57BL mice in the cold, the zeros made an important contribution to the amount of variation. Nevertheless, even with the coefficients calculated in this way, the general picture remains unchanged.

Ashoub, Biggers, McLaren & Michie (1958) have given an example of increased variation in body weight in a heterogeneous stock of TO mice in unfavourable environments: they subjected pregnant females and their young to temperatures of 5° C. or 28° C.; controls were, like ours, kept at 21° C. The young born to the females were weighed at 1, 2, 3 and 4 weeks, and it was the variation in these weights that was found to be increased by the extreme temperatures. By contrast, our mice weighed at 3 weeks (and, in the case of the 'stressed' mice, also at 4, 5, and 6 weeks) showed no significant effect of temperature on variation in body weight. This is in conformity with the fact that the mean weights of the different classes of mice were similar at each age. By contrast, the means in the parameters of reproduction differed widely, and it was here that differences in variance were found. The main effect of the cold on the young inbred mice of our experiments was to kill them, and the dead mice represent the extreme variants. The difference

between our young mice and those of Ashoub and his colleagues may be due either to their use of a different stock or to the higher temperature to which their 'cold-stressed' mice were subjected.

Our F_1 mice, then, showed three major effects of hybridity: (i) heterosis in reproductive performance in both environments; (ii) a greater advantage of hybridity in the less favourable environment; (iii) a greater phenotypic uniformity, especially in the less favourable environment.

From a stockbreeding point of view the value of using hybrid animals was more marked when the animals were in inferior conditions, whether value is measured in terms of numbers of of biomass. This economic attitude to hybrid vigour is, however, superficial. What is the significance of heterosis in terms of the natural-selection process and the genetical mechanism? Discussions of this question have been published by Haldane (1949, 1955), Lerner (1954), Dobzhansky & Levene (1955), Jinks & Mather (1955), McLaren & Michie (1956) and Maynard Smith (1956), and several hypotheses have been advanced to account for the connexion between heterosis and adaptability. Low variance in a given environment and the ability to respond adaptively to a changed environment have been held to be aspects of a single type of process, that is, they have been supposed to reflect an increase in the resources of the genotype: this is thought to confer both improved 'homeorhesis' (Waddington, 1957) in a given environment and also the possibility of alternative developmental paths in different environments. However, heterozygosis does not always produce developmental stability (e.g. Tebb & Thoday, 1954; Jinks & Mather, 1955). It has therefore been suggested—for example, by Jinks & Mather—that superior fitness of heterozygotes, when this is in fact observed, must be a result of natural selection, and need not be attributed to any inherent property of the heterozygous state as such. However, the two hypotheses are not necessarily opposed: it remains possible that the nature of gene action is such that heterozygotes must often tend to be more versatile in their response to widely different environments than homozygotes; yet the extent to which this is true, and the precise form of the heterosis, may be influenced by natural selection.

The data given in this paper support the belief that heterozygosis confers on an organism an increased ability to withstand the effects of environmental hazards on its development; in this regard the marked uniformity of the F_1 mice in the unfavourable environment is especially important. They also suggest a possibility for further study. If heterosis is a result of the action of natural selection on heterozygotes, the more important a process is for physiological adaptation to environmental hazards, the more it should be enhanced by hybridity. This possibility is being studied.

5. SUMMARY

The effect of hybridity on reproduction in mice has been studied in two environmental temperatures: 21° C. ('normal') and −3° C. (unfavourable). Mice of inbred strains A2G and C57BL are maintained as permanent breeding stocks in each of the two temperatures. In each temperature the two strains were crossed,

and the reproduction of F_1 pairs, to the age of 28 weeks, compared with that of the parent strains.

At 21° C. the expected superior fertility of the F_1 mice was found: the number of litters produced was not affected, but there were more young produced per pair. The number of young per pair reared to 3 weeks was about twice the mean of the parent strains at the same temperature.

At −3° C. the difference was greater. The F_1 pairs produced more and larger litters than the parent strains, and deaths between birth and weaning at 3 weeks were few. The number of young per pair reared to 3 weeks was nearly five times the mean of the parent strains. Part of the superiority of the F_1 mice, at both temperatures, was due to the fact that they began to breed earlier.

Despite the larger litters produced by the F_1 pairs, at 21° C. the mean weight of the F_2 young at the age of 3 weeks was intermediate between those of the parent strains. At −3° C. it was the same as that of the heavier parent strain, namely, A2G.

As a further test of resistance to cold, F_1 mice born in the warm environment were transferred to the cold at the age of 22 days and there placed each alone in a cage, with nesting material. In these conditions they had a higher survival rate than the young of either of the parent strains. They also grew faster than the A2G mice.

The F_1 mice were not only more fertile than the inbred mice, but also more uniform in breeding performance. This difference was especially marked in the less favourable environment.

These observations are in conformity with the view that heterosis is a consequence of heterozygosis; and that it depends on an enhanced ability to withstand disturbances of developmental and physiological equilibria.

We are grateful to the Medical Research Council for financial help. We are also indebted to J. Keys for the care he has taken of the mice.

REFERENCES

Ashoub, M. R., Biggers, J. D., McLaren, A. & Michie, D. (1958). The effect of the environment on phenotypic variability. *Proc. roy. Soc.* B, **149**, 192–203.

Barnett, S. A. (1956). Endothermy and ectothermy in mice at −3° C. *J. exp. Biol.* **33**, 124–133.

Barnett, S. A. & Coleman, E. M. (1960). The effect of low environmental temperature on the reproductive cycle of female mice. *J. Endocrin.* (in the press).

Barnett, S. A., Coleman, E. M. & Manly, B. M. (1960). Mortality, growth and liver glycogen in young mice exposed to cold. *Quart. J. exp. Physiol.* (in the press).

Barnett, S. A. & Manly, B. M. (1954). Breeding of mice at −3° C. *Nature, Lond*, **173**, 355.

Barnett, S. A. & Manly, B. M. (1956). Reproduction and growth of mice of three strains, after transfer to −3° C. *J. exp. Biol.* **33**, 325–329.

Barnett, S. A. & Manly, B. M. (1958). Adaptation to cold in young mice. *Physiol. Bohemoslov.* **7**, 19–28.

Barnett, S. A. & Manly, B. M. (1959). Effect of low environmental temperature on the breeding performance of mice. *Proc. roy. Soc.* B, **151**, 87–105.

BUTLER, L. (1958). The inheritance of litter size, body weight, and variability, in a cross between inbred strains of mice. *Canad. J. Zool.* **36,** 969–983.

CHAI, C. K. (1956). Analysis of quantitative inheritance of body size in mice. 2. Gene action and segregation. *Genetics,* **41,** 165–178.

DOBZHANSKY, TH. (1950). Genetics of natural populations. 19. Origin of heterosis through natural selection in populations of *Drosophila pseudo-obscura*. *Genetics,* **35,** 288–302.

DOBZHANSKY, TH. & LEVENE, H. (1955). Genetics of natural populations. 24. Developmental homeostasis in natural populations of *Drosophila pseudo-obscura*. *Genetics,* **40,** 797–808.

DONALD, H. P. (1955). Controlled heterozygosity in livestock. *Proc. roy. Soc.* B, **144,** 192–203.

FALCONER, D. S. & KING, J. W. B. (1949). Large and small mice. *Heredity,* **3,** 380.

GATES, W. H. (1925). Litter size, birth weight, and early growth rate of mice (*Mus musculus*). *Anat. Rec.* **29,** 183–193.

GRÜNEBERG, H. (1954). Variation within inbred strains of mice. *Nature, Lond.*, **173,** 674–676.

HALDANE, J. B. S. (1949). *Ric. Sci.*, Suppl. 54–55.

HALDANE, J. B. S. (1954). *The Biochemistry of Genetics*. London: Allen & Unwin.

HALDANE, J. B. S. (1955). On the biochemistry of heterosis, and the stabilization of polymorphism. *Proc. roy. Soc.* B, **144,** 217–220.

JINKS, J. L. & MATHER, K. (1955). Stability in development of heterozygotes and homozygotes. *Proc. roy. Soc.* B, **143,** 561–578.

LERNER, I. M. (1954). *Genetic Homeostasis*. Edinburgh: Oliver & Boyd.

MCLAREN, A. & MICHIE, D. (1954). Are inbred strains suitable for bio-assay? *Nature, Lond.*, **173,** 686–688.

MCLAREN, A. & MICHIE, D. (1956). Variability of response in experimental animals. *J. Genet.* **54,** 440–455.

MARSHAK, A. (1936). Growth differences in reciprocal hybrids and cytoplasmic influence on growth in mice. *J. exp. Zool.* **72,** 497–510.

MAYNARD SMITH, J. (1956). Acclimatization to high temperatures in inbred and outbred *Drosophila subobscura*. *J. Genet.* **54,** 497–505.

TEBB, G. & THODAY, J. M. (1954). Stability in development and relational balance of X-chromosomes in *Drosophila melanogaster*. *Nature, Lond.*, **174,** 1109.

THODAY, J. M. (1953). Components of fitness. *Symp. Soc. exp. Biol.* **7,** 96–113.

WADDINGTON, C. H. (1942). Canalization of development and the inheritance of acquired characters. *Nature, Lond.*, **150,** 563–565.

WADDINGTON, C. H. (1957). *The Strategy of the Genes*. London: Allen & Unwin.

Genet. Res., Camb. (1960), **1**, *pp.* 39–49
Printed in Great Britain

The birth weight of rabbits born after heterospermic insemination

By R. A. BEATTY
Agricultural Research Council Unit of Animal Genetics, Institute of Animal Genetics, Edinburgh, 9

(*Received* 4 *May* 1959)

INTRODUCTION

Insemination with mixed semen from two or more sires has been called *heterospermic insemination*. The more usual mode of insemination, using semen from one sire at a time, may be called *homospermic insemination*. During an experiment with the rabbit (Beatty, 1955, 1957*a*), it was noted that when genetically large and genetically small offspring were born in the same litters after heterospermic insemination, the average difference in birth weight between them was greater than when they were conceived in different litters after homospermic insemination. This accentuation of the difference in birth weight was termed the *enhancement effect* in relative birth weight. An approximate test of significance suggested that the effect was real. In a search for supporting evidence, an analogous situation was explored in mammalian twins, where the genetically large and genetically small offspring are the male and female young respectively. Like-sexed twin pairs are analogous to litters born after homospermic insemination. Unlike-sexed twin pairs are analogous to mixed litters born after heterospermic insemination. An enhancement effect was, indeed, apparent in human twins and in those from the sheep (Beatty, 1956). An independent and more detailed study of sheep twins by Donald and Purser (1956) showed the same effect.

The present work is, primarily, an independent confirmation of the reality of the enhancement effect after heterospermic insemination in the rabbit. As in the earlier work with rabbits, it has been possible also to test the claim that heterospermic insemination gives superior practical results in the form of 'heterospermic vigour' among the offspring, evidenced by greater birth weight (see, e.g., Kushner, 1954). However, once again, no real 'heterospermic vigour' could be demonstrated. The sampling structure of the earlier experiment was not well defined, and no formal quantitative integration with the present results has been attempted.

The enhancement effect is of interest in showing that the size of an animal at birth is influenced by the genotype of its litter-mates. Further, it constitutes evidence of competition among offspring *in utero*.

MATERIAL AND METHODS

Two sires were used. ♂L was a Flemish Giant, weighing 4·5 kg., and genetically *AA BB CC DD RR*. ♂S was of indeterminate breed, weighed 2·5 kg., and was genetically *aa B cc^h D rr*. The two males were known to give offspring differing

considerably in mature weight, and named Large and Small offspring. The sixty-eight females, from a stock of indeterminate breed averaging about 3·0 kg. in weight, were each used once only, and were of genetic constitution *cc* and/or *rr*. When the female was *cc* (homozygous for the recessive character albino), offspring of ♂L had dark eyes (*Cc*), while those of ♂S were unpigmented (either *cc* or cc^h). When the female was *rr* (homozygous for rex, a recessive hair-waving factor), offspring of ♂L had normal whiskers (*Rr*), whereas offspring of ♂S had waved whiskers (*rr*). At birth, offspring could therefore be traced to their sires by eye-colour and/or the appearance of their whiskers. Artificial insemination was carried out by the method of Walton (1945). In heterospermic insemination, semen of the two sires was mixed in proportions that yielded roughly equal numbers of offspring from each sire. 0·85% NaCl was used as semen diluent. Inseminates were 1 ml. in volume and contained a total of *ca.* 5–30 million spermatozoa. All inseminations were artificial, excepting that eleven additional litters born after natural mating were available and were classed among the homospermic litters. Females, as they became available for the experiment, were allocated to the different classes of insemination so that running totals of roughly equal numbers of litters were obtained from ♂L only, from ♂S only, and from mixed semen.

Hutches were examined for litters from 9 a.m. to 5 p.m. daily. Offspring found were recorded as born on that day. Their birth weight was scored, to the nearest gramme, as their weight at the time they were found. Five damaged offspring, and a miniature still-birth, were dealt with as described in Table 1.

The following classes of litter were obtained. It may be noted that 'heterospermic litters' are all litters born after heterospermic insemination, while 'mixed litters' are the class of 'heterospermic litters' in which offspring by both sires are present in the same litter.

Type of insemination	Sire of offspring	Convention for naming litter		Number of litters	Number of offspring
Homospermic L	♂L	Homospermic L	Homospermic	18	87
Homospermic S	♂S	Homospermic S		23	116
Heterospermic	♂L	Heterospermic L	Heterospermic	2	7
	♂S	Heterospermic S		3	13
	♂♂L+S	Mixed litter		22	68 by ♂L 76 by ♂S

The object of study was birth weight, in the form of the litter average of the logarithmic birth weights of the class of offspring under consideration, and it was desired to adjust for certain attributes of each litter. The remainder of this section describes the biometrical methods employed.

Homospermic insemination						Heterospermic insemination					
Sire L			Sire S			Sire L			Sire S		
Birth weight	Number in litter	Gestation period (days)	Birth weight	Number in litter	Gestation period (days)	Birth weight	Number in litter	Gestation period (days)	Birth weight	Number in litter	Gestation period (days)
78	4	33	59	2	32	68	4		—	—	32
41	2	28	60	4	33	54	3		—	—	32
71	3	34	50	6	32	—	—		46	5	31
66	2	33	46	8	31	—*	—		46*	7	31
85	1	33	55	3	32	—*	—		63*	1	33
96	2	33	50	5	31	27	2		23	2	28
37	9	31	50	7	31	62	2		56	2	32
60	4	33	58*[c]	3	35	28	6		29	1	27
31[c]	5	30	65*[c]	2	32	58	2		44	7	32
45	9	31	44*	5	32	74	1		60	2	32
55	7	32	50*	5	31	71	1		65	2	32
52	6	32	36*[c]	5	30	62	5		41	4	31
58*	5	32	56*	6	33	63	3		49	6	31
69*	5	32	46*	5	30	69	4		52	1	33
56*[c]	5	35	48*	4	30	60	3		54	4	31
69*	4	33	55*	5	30	52	5		46	2	31
76*	4	32	47*	4	32	60	6		54	1	33
26*[c]	10	30	40*	8	31	58	4		48	5	32
			62*	7	31	59	4		49	3	31
			51*	6	31	62	6		60	1	33
			52*	5	31	45	2		35	3	32
			50*	5	31	38	4		42	5	32
			50*	6	32	46*	1		51*	5	32
						46*	2[d]		36*	6	30
						39*	2		35*[b]	7	31
						66*	1		65*	3	31
						66*	2		55*	4	31

[a, b] If the original mean logarithmic birth weights (to two places of decimals) are to be reconstituted, double rounding-off error is avoided by taking 1·41 as the logarithm at *a* and 1·55 as the logarithm at *b*.

[c] One offspring in each litter damaged and unweighable, and given a substitute birth weight equal to the mean of its litter-mates.

[d] A still-birth weighing 2·5 g. was excluded when computing mean birth weight and proportion of Large offspring in the litter, but is included in the 'number in the litter'.

* Two classes of dam were used, differing in body weight. Litters from the lighter class are marked with an asterisk.

Birth weight was analysed after $\log_{10}$ transformation of the birth weight of each offspring. This was known to improve homogeneity of variance within the litter. It also brought birth weight into nearly linear relationship with an important variable, the number of offspring per litter (Venge, 1950). Further, it was felt that relative birth weight of the two types of offspring, as measured by a difference between logarithms, would be more nearly a constant than the actual difference in grammes. The following averages of the logarithmic birth weights were studied, and formed the dependent variables in the multiple regressions described later: Y_1, mean logarithmic birth weight per litter in homospermic litters (data from homospermic L + homospermic S litters); Y_2, mean logarithmic birth weight per litter in heterospermic litters (data from heterospermic L + heterospermic S + mixed litters); Y_3, mean logarithmic birth weight per litter of Large offspring in heterospermic litters (data from heterospermic L + mixed litters); Y_4, mean logarithmic birth weight per litter of Small offspring in heterospermic litters (data from heterospermic S + mixed litters); Y_5, mean logarithmic birth-weight difference in mixed litters (mean logarithmic birth weight of Small offspring subtracted from mean logarithmic birth weight of Large offspring in the twenty-two mixed litters).

The following data recorded for each litter formed the independent variables in the multiple regressions described below, the means being the grand experimental means (weighted by number of offspring per litter): X_1, gestation period in days, mean 31·4; X_2, number of offspring in litter, mean 6·29; X_3, maternal weight, entered as a 'dummy variate' with values of 100 for a heavier lot of females and 0 for a lighter lot, mean 58; X_4, percentage of Large offspring per litter, entered as either 100% or 0% for homospermic L and homospermic S litters respectively, and varying from 100% to 0% in the heterospermic litters, mean 44%. These grand means will be referred to as 'standard gestational conditions' to which birth weights could be adjusted before comparison.

Multiple regressions of $Y_1 \ldots Y_5$ were then carried out on the four independent variables $X_1 \ldots X_4$. The regressions of $Y_1 \ldots Y_4$ were weighted by the number of young under consideration per litter. The regression of Y_5 was weighted by $n_1 n_2/(n_1+n_2)$, n_1 and n_2 being respectively the numbers of Large and Small offspring per litter. The multiple regressions of $Y_1 \ldots Y_4$ were highly significant and accounted for about half the sum of squares of Y. This was mainly due to the large sums of squares taken out by the partial regressions on X_1 and X_2. The partial regression coefficients on X_3 and X_4 were all small and non-significant. In using these multiple-regression equations for adjusting birth weights, the means of $Y_1 \ldots Y_4$ were little affected by setting $X_1 \ldots X_4$ to 'standard gestational conditions', but a considerable diminution in the standard errors of predicted Y was achieved. Neither the multiple regression of Y_5 nor any of its partial-regression coefficients even approached significance, and it was therefore concluded that the *relative* birth weight of Large and Small offspring in mixed litters bore little or no relation to the values of $X_1 \ldots X_4$. No adjustment of Y_5 to 'standard gestational conditions' was deemed necessary. The mean logarithmic birth-weight difference

between Large and Small offspring in mixed litters was therefore taken as the simple weighted mean of the 22 differences.

The relationship between birth weight and number of offspring per litter was nearly linear as a result of the logarithmic transformation of birth weight. Other relationships implicit in the multiple regressions were assumed for the present purposes to be approximately linear.

In any multiple regression, it is implicit that variations in the X may cause changes in the value of Y, but that variations in Y do not affect the X. This one-way direction of cause and effect certainly applies for X_3, whose values were set wholly by the choice of dam, while X_4 was determined largely by the proportions in which semen from each male were arranged to contribute to the final inseminate. Variations in X_1 and X_2 were assumed to be determined mainly by the particular constitution of each dam (see Venge, 1950; Beatty, 1957*a*). Possible effects of Y on X_1, X_2 and X_4 were assumed to be negligible.

The five multiple regressions served the purpose of integrating the information of this experiment in convenient form for computing the various weighted logarithmic means and standard errors described in the Results section. Antilogarithms of the logarithmic means yielded weighted geometric-mean birth weights in grammes. Antilogarithms of mean differences yielded geometric-mean ratios. It has not been felt necessary to repeat in what follows that all data are weighted. Analyses have been conducted only so far as to bring out the main points of the experiment.

RESULTS

A summary of the original data is given in Table 1.

1. *Comparison of birth weight in homospermic and heterospermic litters; absence of 'heterospermic vigour'*

Birth weights in homospermic and heterospermic litters were compared first from the crude data, i.e. from the mean logarithmic birth weights of all homospermic and of all heterospermic offspring. As shown in the right-hand column of Table 2, in the form of geometric means, there is little difference between the homospermic and heterospermic litters, the heterospermic offspring averaging 1·1 g. *less* than the homospermic offspring: this difference was found to be non-significant.

Birth weights were then analysed after adjustment to 'standard gestational conditions'. As shown in Table 2, the difference between the mean logarithmic birth weights of homospermic and heterospermic litters scarcely exceeds its standard error and is obviously non-significant. These logarithmic means have been translated into geometric means in the central column of figures of the Table; heterospermic offspring average 2·3 g. more than homospermic litters.

In the crude data and in the adjusted data, there is therefore no evidence of any real effect of heterospermic insemination on the birth weight of the heterospermic litter as a whole.

Table 2. *Birth weights of offspring in homospermic and heterospermic litters*

Type of litter	Birth weight adjusted to 'standard gestational conditions': Logarithmic mean ± standard error	Geometric mean (g.)	Unadjusted geometric mean birth weight (g.)
a. Heterospermic	1·703 ± 0·0158 (22 d.f.)	50·4	48·7
b. Homospermic	1·682 ± 0·0124 (36 d.f.)	48·1	49·8
Difference, *a* − *b*	+0·021 ± 0·0200 (58 d.f.)		

The column of logarithmic means and standard errors is derived from the multiple regressions of Y_1 and Y_2, with $X_1 \ldots X_4$ set at the grand experimental means. The antilogarithm of the logarithmic mean gives the geometric mean. The unadjusted geometric means are derived from logarithmic means of the crude data without use of multiple regression.

2. *Difference between birth weights of Large and Small offspring in homospermic versus heterospermic litters; confirmation of the enhancement effect*

From the crude data (geometric means of all offspring in the classes of offspring under consideration, not adjusted by multiple regression), it was found that the difference in birth weight between Large and Small offspring (Large − Small) was −0·09 g. in homospermic litters, but +7·65 g. in heterospermic litters. The difference between these differences (+7·74 g.) represents a positive enhancement effect, as was also found in the more detailed analysis given below.

The mean logarithmic difference in birth weight between Large and Small offspring (Large − Small) in homospermic litters, independent of gestation period, number of offspring per litter, and maternal weight, was given by the partial regression coefficient of Y_1 on X_4. Its value was −0·008 ± 0·0260 (36 d.f.). This indicates a slight estimated inferiority in the weight of Large offspring, but the difference is clearly non-significant in comparison with its large standard error, and could be consistent with a real difference that was either positive or negative.

The mean logarithmic difference in birth weight between Large and Small offspring (Large − Small) in mixed heterospermic litters was obtained directly from the 22 logarithmic differences, as outlined on p. 42, use of the non-significant multiple regression having been considered unnecessary. Its value of +0·068 ± 0·0124 (21 d.f.) was highly significant ($P \ll 0{\cdot}001$). The reality of this difference was quite obvious even in the raw data (see Table 1). From the antilogarithm of this mean, it was seen that Large offspring averaged 16·9% heavier than Small offspring at birth, confidence limits being 24·0% and 10·1% at the 0·05 probability level.

The difference between the two mean logarithmic differences measures the enhancement effect. Its value of +0·076 is significantly different from zero, the Behrens-Fisher test (Fisher and Yates, 1948) and the Cochran-Snedecor test (Snedecor, 1956) each yielding a significance level of $P = 0{\cdot}05$–$0{\cdot}01$, and probably

nearer to 0·01 than 0·05. The enhancement effect may therefore be considered a real phenomenon.

3. *Effect of heterospermic insemination on the relative birth weights of Large and Small offspring*

The difference in birth weight between Large and Small offspring is enhanced in mixed litters. Is this due to increased weight of Large offspring, or to decreased weight of Small offspring, or to both factors? The question was investigated after adjustment of birth weights to 'standard gestational conditions' of gestation period, number of offspring per litter, and maternal weight. As shown in Table 3,

Table 3. *Mean geometric birth weights of Large and Small offspring in homospermic and heterospermic litters, as at 'standard gestational conditions' of gestation period, number of offspring per litter, and maternal weight*

Type of offspring	Type of litter: Homospermic	Type of litter: Heterospermic	Difference
Large	47·6 g.	54·6 g.	+7·0 g.
Small	48·4 g.	48·1 g.	−0·3 g.

Means for homospermic litters are derived from the multiple regression of Y_1, with $X_1 \ldots X_3$ set at the grand experimental means, and X_4 set at 100 (for Large offspring) or at 0 (for Small offspring). Means for heterospermic litters are derived from the multiple regressions of Y_3 and Y_4, with $X_1 \ldots X_4$ set at the grand experimental means. Tests carried out on the logarithmic figures showed that the difference of +7·0 g. was nearly significant at the $P = 0{\cdot}05$ level, while the difference of −0·3 g. was not significant.

heterospermic insemination brought about little estimated change in the birth weight of Small offspring, the decrease of 0·3 g. being found non-significant. The estimated effect on Large offspring was, however, a considerable increase in birth weight (7·0 g.) whose significance was found to lie close to the conventional limit of $P = 0{\cdot}05$.

The experiment was evidently on too small a scale to permit any firm general conclusions in this section. There is suggestive evidence that Large offspring increase in weight after heterospermic insemination. If we accept the implication of the results on p. 43, i.e. that there is no real increase in the birth weight in the heterospermic litter as a whole, it would follow that any increase in the weight of Large offspring must be compensated for by a decrease in the weight of Small offspring. But the reality of this decrease has not been established by a direct test.

DISCUSSION

The enhancement effect seems now to be fairly well established. It may be described as follows. When genetically large and genetically small offspring are born in the same litter, the average difference in birth weight between them is

greater than when they are conceived in litters all of one genetic type. This generalization applies to all the existing data, i.e. to the previous experiment with heterospermic insemination in the rabbit (Beatty, 1957*a*), to the present confirmatory experiment with the rabbit, and also to the analogous 'natural experiments' of human and sheep twins mentioned in the Introduction. These effects on development reflect a complex genotype-environment interaction. An offspring develops under the influence of (*a*) its own gene complement, (*b*) the gene complement of the dam, mediated by maternal and possibly by cytoplasmic effects, and (*c*) the various gene complements of its uterine litter-mates, mediated by what appears to be intra-uterine competition. The litter-mates may be regarded as a part of the prenatal environment determined by gene complements that are not the same as those determining the maternal soma.

In the present experiment and in the previous one (Beatty, 1957*a*) it was known that homospermic offspring of the larger sire had a greater *mature* weight than those of the smaller sire. But it has not yet been proved in homospermic litters that the offspring of the larger sire have a significantly greater *birth* weight than those of the smaller sire. The reason for this is, perhaps, that the comparison is difficult to make because it is involved with the high variance between dams. In heterospermic mixed litters, on the other hand, a much more efficient comparison can be made, within the litter. Any accurate comparison of birth weights in homospermic litters demands either larger numbers of observations, or else a more efficient sampling design. Correction for the covariance of the weight of the dam might also be made more efficiently than in the present work. In the meantime, it will be assumed that the larger sires have the larger offspring at birth in homospermic litters. Demonstration of the enhancement effect does not depend on the validity of this assumption.

By contrast with homospermic litters, it has proved quite easy to show in mixed litters born after heterospermic insemination that offspring of a genetically and phenotypically larger sire have a significantly greater birth weight than those of a genetically and phenotypically smaller sire. This was found in the present work and in the previous investigation (Beatty, 1957*a*), and can be computed even in the three mixed litters described by Kopeć (1923). Genomes affecting body weight in a given sense must begin to exert their effect in the embryo or foetus. Further, since the offspring of a heterospermic litter are conceived at the same time and born at the same time, the genetic effect on birth weight must mean a genetically determined prenatal effect on growth rate.

The enhancement effect can be explained in terms of embryonic competition, with the larger type of embryo gaining an undue advantage at the expense of the smaller type. In the earlier data, however (Beatty, 1957*a*, and the references to work on mammalian twins given in the Introduction), it is not possible to eliminate an alternative explanation (see Donald and Purser, 1956) concerned with the gestation period. This begins with the null hypothesis that Large and Small offspring each have their typical average growth rate, irrespective of whether they are born in homospermic or heterospermic litters. In heterospermic litters, the

time of birth is necessarily the same for both kinds of offspring in a litter, and the difference in their growth-rates would be immediately revealed as a difference in birth weight. In homospermic litters, the Large offspring, although growing fast, might for some reason be born earlier, and/or the slow-growing Small offspring might be born late. Thus, in homospermic litters, the potential difference in birth weight between Large and Small offspring might be largely annulled by changes in the gestation period. This explanation is, however, eliminated by the present work, in which comparisons of birth weight have been made *as at a constant gestation period* for the whole experiment, and an enhancement effect nevertheless resulted. There seems therefore no remaining obstacle to interpreting the enhancement effect as an example of competition among embryos *in utero*, and indeed as evidence of such competition. The question of exactly what substance or substances are being competed for is outside the scope of this paper.

In the previous experiment (Beatty, 1957*a*), the enhancement effect was attributable primarily to decreased weight of Small offspring—but average birth weight in the heterospermic litter as a whole was slightly low. In the present work, the enhancement effect was attributable primarily to increased weight of Large offspring—but average birth weight in the heterospermic litter as a whole was slightly high. These data suggest that the enhancement effect in mixed heterospermic litters is due both to an increase in the birth weight of Large offspring and decrease in the birth weight of Small offspring. This conclusion is provisional. If correct, it would agree with an analogous trend mentioned by Donald and Purser (1956) in their work on sheep twins.

We may now consider the 'heterospermic vigour' said to be evidenced by an increased average birth weight in litters born after heterospermic insemination (e.g. Kopeć, 1923; and a large body of evidence, mainly Russian, summarized by Kushner, 1954). In my first test experiment (Beatty, 1957*a*), average birth weight after heterospermic insemination was slightly *less* than after homospermic insemination from either sire. In the present work, with data adjusted to 'standard gestational conditions', the average birth weight in heterospermic litters was slightly but non-significantly greater than the average birth weights in the two kinds of homospermic litter. Thus, there has been no confirmation of the reality of 'heterospermic vigour' in the average birth weight of the litter as a whole. However, when the enhancement effect is considered, it could be said in a special sense that 'heterospermic vigour' has occurred, because one type of offspring (Large offspring) was, apparently, unduly large in heterospermic litters. But this appeared to be compensated for by a negative 'heterospermic vigour' among the Small type offspring, so that the litter as a whole did not have an unusual average birth weight. It is not clear whether the Russian workers would, in fact, use the term 'heterospermic vigour' in this special sense.

Studies of competition among organisms are at a much more advanced stage in plants, where the type of interaction is well known to alter with the particular genetical and environmental conditions. Further, Sakai (1955) points out as an additional complication that competitive ability in plants is not necessarily a

function of size or yield. In mammals, it would be an over-simplification of the situation to expect, under all circumstances, only one kind of interaction between organisms grown under competitive conditions in a microcosm such as the uterus. In pursuing the type of work suggested by the Russian experiments on mammals, the question should be 'What are the different effects of heterospermic insemination under various circumstances?' rather than 'What is the effect of heterospermic insemination?' Offspring in heterospermic litters seem to interact co-operatively in the Russian experiments, but competitively in mine; neither finding is necessarily a disproof of the other. I must admit, however, that I have not been encouraged in a general sense by the results of my three attempts to confirm claims made by the Michurinist school of biology—i.e. in the present work and in the earlier work with heterospermic insemination (Beatty, 1957*a*), and in a search for somatic inconstancy in the chromosome number of mice (Beatty, 1957*b*). Other aspects of 'heterospermic vigour' have also failed to receive general confirmation in the West (see, e.g., Campbell and Jaffe, 1958). But, although the reality of 'heterospermic vigour' may well be viewed with reserve, the proposition that all the Russian work on this subject is always wrong may be viewed with even greater reserve.

SUMMARY

1. Two rabbit sires were used for insemination of sixty-eight females. Insemination was either homospermic (one sire at a time) or heterospermic (mixed semen from the two sires). Each offspring could be traced to its sire by genetic marks. The sires differed in weight and were known to give offspring differing in mature weight and named Large and Small offspring. The object of study was the birth weight of these offspring in logarithmic transformation.

2. After heterospermic insemination, there was no evidence of any real 'heterospermic vigour' in the average birth weight of the litter as a whole.

3. After heterospermic insemination, the difference in birth weight between the two kinds of offspring was accentuated (enhancement effect). This confirms a previous experiment.

4. The enhancement effect is ascribed to competition among embryos. It is not attributable to postulated changes in the gestation period. It appears to arise from an increase in the birth weight of Large offspring, together with a possible decrease in the birth weight of Small offspring. These changes in birth weight are attributable to changes in prenatal growth rate.

5. The bearing of this work on Russian experiments with heterospermic insemination is discussed.

I am grateful to Prof. C. H. Waddington for helpful interest and facilities. Dr D. J. Finney explained some difficult statistical points, and Dr D. S. Falconer was kind enough to criticize the manuscript. The work was carried out with technical assistance from Mr A. M. Dalrymple, and with further assistance from Miss A. G. Stenhouse and Mrs M. Lawson.

REFERENCES

BEATTY, R. A. (1955). *Res. Rep. 1952–54, Inst. Anim. Genet., Edinb.*, edit. C. H. Waddington, p. 25.

BEATTY, R. A. (1956). Relation between genetic constitution of an offspring and weight of its litter-mates. *Nature, Lond.*, **178**, 48–49.

BEATTY, R. A. (1957 *a*). A pilot experiment with heterospermic insemination in the rabbit. *J. Genet.* **55**, 325–347.

BEATTY, R. A. (1957 *b*). Chromosome constancy in the corneal epithelium of the mouse. *Chromosoma*, **8**, 585–596.

CAMPBELL, R. C. & JAFFE, W. P. (1958). The motility of mixed semen. *J. agric. Sci.* **50**, 64–65.

DONALD, H. P. & PURSER, A. F. (1956). Competition *in utero* between twin lambs. *J. agric. Sci.* **48**, 245–249.

FISHER, R. A. & YATES, F. (1948). *Statistical Tables.* London and Edinburgh: Oliver & Boyd.

KOPEĆ, S. (1923). On the offspring of rabbit-does mated with two sires simultaneously. *J. Genet.* **13**, 371–381.

KUSHNER, K. F. (1954). (The effect of heterospermic insemination in animals and its biological nature.) *Izvestia Akad. Nauk. SSSR*, Ser. Biol., no. 1, p. 32. (As in private translation by D. Michie and A. McLaren.)

SAKAI, K.-I. (1955). Competition in plants and its relation to selection. *Cold Spr. Harb. Symp. quant. Biol.* **20**, 137–157.

SNEDECOR, G. W. (1956). *Statistical Methods*, 5th ed. Iowa State College Press.

VENGE, O. (1950). Studies of the maternal influence on the birth weight in rabbits. *Acta zool. Stockh.*, **31**, 1–148.

WALTON, A. (1945). *Notes on the technique of artificial insemination.* London: The Holborn Surgical Instrument Co. Ltd.

Genet. Res., Camb. (1960), **1**, *pp.* 50–58
With 3 text figures
Printed in Great Britain

How pure are our inbred strains of mice?

BY M. S. DEOL, H. GRÜNEBERG, A. G. SEARLE* AND G. M. TRUSLOVE

Medical Research Council Group for Experimental Research in Inherited Diseases, University College, London

(*Received* 25 *May* 1959)

For many years, the genetic homogeneity of inbred strains of animals and plants has been taken for granted on the strength of the mathematical theory of inbreeding. More recently, evidence has been accumulating that inbred strains tend to break up into genetically differentiated sublines following a course of inbreeding which, according to theory, should have resulted in the fixation of virtually all the initial genetic variance. A systematic study of skeletal variation in a British substrain of the inbred strain C57BL of the mouse by the present authors (1957) showed that in seven sublines which diverged from each other following at least forty generations of brother-sister mating, thirteen out of twenty-seven skeletal variants studied occurred with about the same frequency in all sublines; the remaining fourteen variants occurred in frequencies which differed between sublines or groups of sublines. Some American sublines of the same inbred strain which had been separated from each other for a longer period had drifted apart to a greater extent (Carpenter, Grüneberg & Russell, 1957). For any one variant, subline differences arise by sudden, discontinuous steps. In principle, these may be due to the segregation of residual gene differences dating from the origin of the C57BL strain, it being assumed that physiological advantages inherent in heterozygotes greatly delayed the fixation of genes in homozygous condition; or to mutations which arose following the establishment of a genetically homogeneous strain. All the available evidence led to the conclusion that the subline differences observed are the result of mutation rather than segregation. However, it would still be of interest to know whether a freshly arisen mutation is fixed promptly in an inbred strain according to theory, or whether it tends to linger in heterozygous condition for lengthy periods before it ultimately becomes fixed. In the absence of genetic variance there will be no correlation between parents and offspring for any given character. The existence and magnitude of parent-offspring correlations is a measure of the genetic variance present in an inbred strain.

Three inbred strains of mice were available for investigation, C57BL/Gr, A/Gr and CBA/Gr. Partial pedigrees of the brother-sister pairs whose offspring were examined are given in Figs. 1–3. Much scope for the differentiation of sublines was present in the case of C57BL, less in A, and hardly any at all in CBA. The

* Present address: Medical Research Council, Radiobiological Research Unit, Harwell, Didcot, Berks.

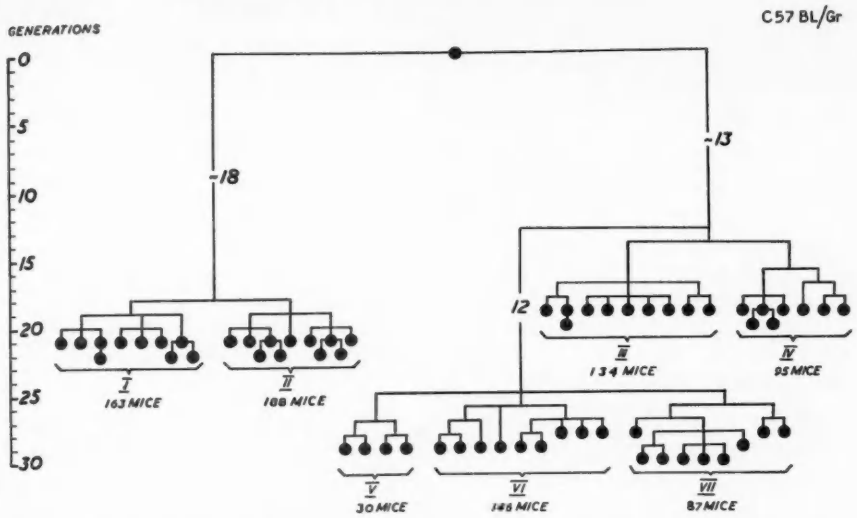


Fig. 1. Partial pedigree of the C57BL/Gr strain. The last common pair of ancestors of the seven sublines (the 'O' generation) lived in 1941 and was preceded by at least forty generations of brother-sister matings. The black circles represent such pairs which produced the young whose skeletons have been examined. From Deol, Grüneberg, Searle & Truslove (1957), by permission of the Wistar Press.

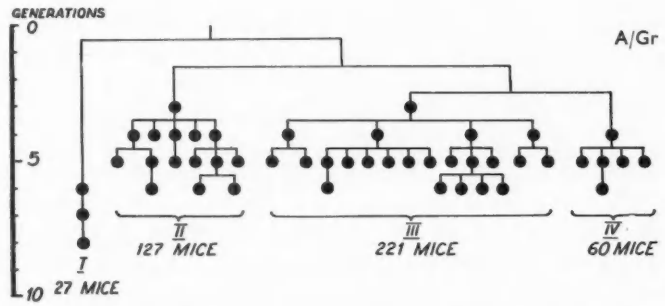


Fig. 2. Partial pedigree of the A/Gr strain. The 'O' generation lived in 1948 and was preceded by at least fifty generations of brother-sister matings.

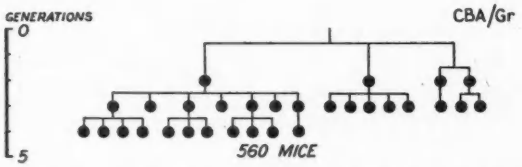


Fig. 3. Partial pedigree of the CBA/Gr strain. The 'O' generation lived in 1948 and was preceded by at least fifty generations of brother-sister matings.

analysis of C57BL has been confined to sublines I–IV; sublines V–VII had to be omitted as the parents of the various sibships were not available for study. The characters studied included some thirty skeletal variants, most of which have been described by the present authors in a series of papers under the general title 'Genetical studies on the skeleton of the mouse' in the *Journal of Genetics*, vols. 50–55, 1950–57. Most variants are all-or-none characters or can be treated as such, giving the possibility of four different types of mating:

	♂	♀
(1)	−	−
(2)	−	+
(3)	+	−
(4)	+	+

Where the variants form a minority of the strain (Nos. 1–12, 15, 18, 20–23 and 25–27 in Table 1), matings of types 2–4 have been pooled, with type 4 often not represented at all. Where the variants form the majority of the strain (Nos. 14, 16, 17, 19 and 29), matings of types 1–3 have been pooled, with type 1 often not represented. With character No. 29 (size of the processus spinosus of Th II), which is a continuous variant, large and medium (+++ and ++) and small and absent (+ and −) processes have been pooled in C57BL and CBA, but in A, in which the larger processes are virtually absent, the line has been drawn between + and −. For similar reasons the pooling of classes for the same variant often differs from strain to strain (Tables 1–3); the actual frequencies of the characters thus cannot be deduced from these tables, but may be obtained from the original papers. No. 24 (foramina transversaria imperfecta) is a meristic character; on each of the cervical vertebrae C III–C VI, one or both foramina transversaria may be open gutters, so that any one individual may have from 0 to 8 foramina open. In this case, matings in which the combined parental count was 5 or less (average 4·00) were compared with matings in which the combined parental count was 6 or over (average 7·93); in the offspring, individuals with three or less open foramina were classified as '−', while animals with four or more open foramina were classified as '+'. Sublines have been treated separately wherever they differ significantly from each other in the incidence of a variant; to pool such different sublines would, of course, lead to spurious parent-offspring correlations: indeed, the existence of genetically differing sublines was first noticed in our C57BL strain through such a spurious parent-offspring correlation. In Nos. 15, 24, 26 and 27 of Table 1, some sublines have supplied no information, as the parents failed to include affected (or, in the case of No. 24, high-grade) individuals. For the same reason, certain variants (No. 4: interfrontal-frontal fusion; No. 13: dyssymphysis of the processus spinosus of Th II; No. 20*a*: abnormal metoptic roots of the presphenoid; and No. 28: frontal fontanelle) are not included in Table 1, and similarly in Tables 2 and 3.

In this fashion, the data for all available characters in the three inbred strains are presented in the form of 2 × 2 tables in Tables 1–3. In each case, the number of

Table 1. C57BL/10

Parent(s) + Offspring

Parent(s) − Offspring

Table 1. C57BL/6J

No.	Variant	Subline	Parent(s) − Offspring − (a)	Parent(s) − Offspring + (b)	Parent(s) + Offspring − (c)	Parent(s) + Offspring + (d)	d_{exp}	V_d	P
1	Lacrimal-maxilla fusion		259	88	138	44	45·4	22·40	0·76
2	Parted frontals		401	36	86	5	7·1	5·41	0·37
3	Fused frontals		345	15	157	11	8·3	5·37	0·24
5	Squamosal-parietal fusion		281	45	165	26	26·2	14·20	0·95
6	Periotic-occipital fusion		387	37	98	6	8·5	6·26	0·32
7	Foramen ovale single		441	29	54	4	3·6	3·03	0·71*
8	Foramen ovale open posteriorly		401	37	85	5	7·2	5·48	0·36
9	Foramen hypoglossi single		71	52	236	169	169·5	23·01	0·91
10	Atlas-axis fusion		366	31	128	13	11·5	7·83	0·60
11	Tub. anterius inflexum of C VI		85	6	61	5	4·6	2·51	0·81
12	Dyssymphysis of Th I		367	96	55	18	15·5	10·58	0·45
14	Interfrontal	I, II	108	170	18	36	33·5	10·68	0·45
	,,	III, IVA	27	74	13	39	38·4	6·67	0·82
	,,	IVB	0	21	2	15	16·1	0·48	0·19*
15	Interparietal-occipital fusion	III, IV	113	8	73	2	3·8	2·25	0·19*
16	Inframaxillary crest	I, II	25	100	51	156	159·6	13·80	0·33
	,, ,,	III, IV	14	83	6	94	89·9	4·51	0·050
17	Alae palatinae	I, II	11	21	74	226	223·2	5·52	0·23
	,, ,,	III, IV	3	39	16	139	140·1	2·89	0·39*
18	Foramen sphenoidale medium		123	91	169	145	140·4	31·52	0·41
19	Processus pterygoideus	I, II	182	72	49	29	23·7	12·67	0·14
	,, ,,	III, IV	32	40	52	72	70·9	11·21	0·73
20	Presphenoid, preoptic sutures	I, II, IV	103	82	124	116	111·8	26·06	0·41
	,, ,, ,,	III	19	29	15	38	36·0	5·68	0·23
21	Accessory mental foramen		69	101	115	244	234·1	26·22	0·054
22	Cervical fusions	I, II, IV	400	16	11	0	0·4	0·39	0·65*
	,, ,,	III	66	13	28	4	4·9	2·98	0·42*
23	Absence of tub. ant. of C VI		157	79	191	110	106·0	30·23	0·46
24	Foramina transversaria imperf.	(excl. IA)	96	106	138	142	143·9	29·37	0·70
25	Dystopia of proc. spin. of Th II		464	14	57	2	1·8	1·52	0·75*
26	Dyssymphysis of Th X	I, II, IV	198	186	23	20	20·7	9·68	0·81
27	Sacralisation of L VI	I, II	213	43	61	14	12·9	8·29	0·70
29	Size of proc. spin. of Th II		14	80	58	301	301·9	9·98	0·76
	Total					2250	2221·4	358·68	

Table 2. *A/Gr*

No.	Variant	Subline	Parent(s) − Offspring − (*a*)	Parent(s) − Offspring + (*b*)	Parent(s) + Offspring − (*c*)	Parent(s) + Offspring + (*d*)	d_{exp}	V_d	P
1	Lacrimal-maxilla fusion		171	92	111	61	60·5	23·77	0·92
2	Parted frontals	I, II, IV	79	36	71	28	29·6	11·20	0·63
	,, ,,	III	111	17	69	24	17·3	8·18	0·018
3	Fused frontals		299	19	110	7	7·0	4·82	1·00
7	Foramen ovale single	II, III, IV	29	55	94	229	225·4	14·09	0·34
	,, ,, ,,	I	3	1	13	10	9·4	0·85	0·45*
9	Foramen hypoglossi single		122	191	48	69	70·7	20·41	0·70
11	Tub. anterius inflexum of C VI		299	37	58	8	7·4	5·50	0·79
14	Interfrontal		335	36	55	9	6·6	5·07	0·29
17	Alae palatinae		377	8	49	0	0·9	0·79	0·38*
18	Foramen sphenoidale medium		4	62	36	333	335·1	4·69	0·24*
19	Processus pterygoideus		67	219	35	114	114·1	17·63	0·99
20a	Presphenoid, metoptic roots abn.		208	86	89	38	37·4	18·47	0·89
21	Accessory mental foramen		37	37	203	157	160·9	15·21	0·31
29	Size of proc. spin. of Th II		123	82	146	81	85·7	25·37	0·36
—	Dyssymphysis posterior of C II		314	53	63	5	9·1	6·65	0·11
—	Arch foramina in Th V		17	68	67	282	281·5	10·69	0·87
	Total					1455	1458·6	193·39	

Table 3. *CBA/Gr*

No.	Variant	Parent(s) − Offspring − (a)	Parent(s) − Offspring + (b)	Parent(s) + Offspring − (c)	Parent(s) + Offspring + (d)	d_{exp}	V_d	P
1	Lacrimal-maxilla fusion	445	8	100	5	2·4	1·94	0·067
2	Parted frontals	46	150	86	276	276·4	23·00	0·94
7	Foramen ovale single	272	177	51	59	46·4	21·59	0·0068
9	Foramen hypoglossi single	87	32	253	114	110·3	18·92	0·39
11	Tub. anterius inflexum of C VI	77	128	139	191	196·8	30·50	0·29
14	Interfrontal	30	169	55	304	304·3	16·56	0·94
17	Alae palatinae	73	48	240	195	190·1	23·33	0·34
18	Foramen sphenoidale medium	7	78	54	421	423·3	7·01	0·40
19	Processus pterygoideus	77	230	46	207	197·4	23·81	0·050
20	Presphenoid, preoptic sutures	8	75	23	450	446·6	3·76	0·080
20a	,, metoptic roots abn.	231	261	38	26	33·0	14·30	0·061
21	Accessory mental foramen	111	67	223	158	153·4	29·23	0·39
27	Sacralisation of L VI	0	41	9	509	509·7	0·60	0·50*
28	Frontal fontanelle	293	146	82	37	39·0	20·67	0·65
29	Size of proc. spin. of Th II	8	135	40	375	379·4	8·38	0·14
—	Absence of third molars	401	99	169	26	35·1	20·72	0·047
	Total				3353	3343·6	264·32	

affected offspring from affected parents (d) is compared with the value expected in the absence of a parent-offspring correlation (d_{exp}), and the variance of the latter (V_d) is also given. This variance is calculated on the basis of the Fisher-Irwin-Yates exact distribution for 2×2 tables. For any one table, with $n = a+b+c+d$ entries, we have

$$d_{exp} = (b+d)(c+d)/n,$$
$$V_d = (a+b)(a+c)(b+d)(c+d)/n^2(n-1).$$

The total expectation for the whole collection of tables is the sum of the expectations in the individual tables, and similarly for the variance V_d, the standard error being the square root of the variance. The P-values given in the last column of Tables 1–3 are based on χ^2 tests or, where appropriate, on Fisher's 'exact' treatment of 2×2 tables; the latter values are marked by an asterisk.

A condensed version of the results is given in Table 4. Treating the three inbred strains separately, the total value of d exceeds its expectation in C57BL and CBA,

Table 4. *Test on sum of* d *values*

	d					
Strain	Observed	Expected	Δ	S.E.$_\Delta$	Δ/S.E.$_\Delta$	P
C57BL	2250	2221·4	+28·6	18·94	1·51	0·13
A	1455	1458·6	−3·6	13·91	0·26	0·79
CBA	3353	3343·6	+9·4	16·22	0·58	0·56
Total	7058	7023·6	+34·4	28·57	1·20	0·23

but falls slightly short of it in A. The difference between observed and expected values is not significant in any single instance; nor is it significant when the data from all the three inbred strains are combined. Considering the sixty-six fourfold tables separately, d is lower than its expectation in thirty-two instances, equals it once, and exceeds it thirty-three times: an embarrassingly close fit.

In Table 5, the fifty-five individual P-values from the χ^2 tests of Tables 1–3 are grouped and compared with their expectations. The observed distribution of

Table 5. *Distribution of fifty-five* P*-values from* χ^2 *tests compared with that expected on a chance basis*

P-value	< 0·01	0·01–0·05	0·051–0·25	0·251–0·50	0·501–0·75	> 75
Observed	1	4	10	18	7	15
Expected	0·55	2·2	11	13·75	13·75	13·75

P-values agrees well with that expected on a chance basis ($\chi^2 = 6{\cdot}666$; $n = 4$; $P = 0{\cdot}16$). The table does not include eleven P-values calculated by means of Fisher's 'exact' method as these represent only one tail of the distribution; they range from 0·19 to 0·75.

In Tables 1–3, there are altogether five values of P of 0·05 or less, the expectation in a sample of sixty-six being 3·3. In none of these cases does a study of the respective pedigrees suggest an incipient differentiation of a subline, and presumably most of them can be regarded as the result of random sampling. However, as in four out of five instances the deviation is in the direction of a parent-offspring correlation, there is a suspicion that perhaps one or the other of these bigger deviations may not have been due to chance alone. But even if one or two out of a total of sixty-six tests were to indicate a parent-offspring correlation, this would not alter the conclusion that the three inbred strains examined are essentially homogeneous with regard to the genes which control the array of skeletal variants used for this investigation. As the number of genes involved is undoubtedly large, the test for genetic heterogeneity here reported is clearly a sensitive one.

As all three inbred strains behave alike, and as there is no reason to suppose that the genes responsible for skeletal variation differ systematically from genes in general, it is legitimate to conclude that inbred strains in the mouse are genetically homogeneous in conformity with the theory of inbreeding, except for the formation of genetically distinct sublines. In addition, the essential absence of genetic variance within the inbred strains confirms our previous conclusion (1957) that the formation of sublines is due to mutation rather than segregation, and it shows that such mutations tend to be fixed promptly in homozygous condition.

Our findings probably have a general application to inbred strains of mice, but clearly not to inbred strains of other organisms. To mention only two exceptions, in the rat (Loeb, King & Blumenthal, 1943; see also Billingham & Silvers, 1959) segregation for histocompatibility genes was still found after 102 generations of brother-sister mating, and in the chicken (Shultz & Briles, 1953; Briles, Allen & Millen, 1957; Cock, 1956; Gilmour, 1959) there is evidence for the persistence of genetic variance in the face of close inbreeding; this is evidently due to the greater vigour and consequently selective advantage of certain heterozygotes as compared with either type of homozygote. The genetic structure of inbred strains thus requires special study in each organism.

SUMMARY

Sixty-six individual tests on an array of skeletal variants showed an essential absence of parent-offspring correlations in the inbred strains C57BL, A and CBA in the mouse. It is concluded that these strains, and inbred strains of mice in general, are genetically homogeneous except for the differentiation of genetically distinct sublines as the result of mutations.

We are indebted to Dr C. A. B. Smith for advice on statistical matters.

REFERENCES

Billingham, R. E. & Silvers, W. K. (1959). Inbred animals and tissue transplantation immunity. *Transpl. Bull.* **6**, 399–406.

Briles, W. E., Allen, C. P. & Millen, T. W. (1957). The B blood group system in chickens. I. Heterozygosity in closed populations. *Genetics*, **42**, 631–648.

CARPENTER, J. R., GRÜNEBERG, H. & RUSSELL, E. S. (1957). Genetical differentiation involving morphological characters in an inbred strain of mice. II. American branches of the C57BL and C57BR strains. *J. Morph.* **100**, 377–388.

COCK, A. G. (1956). Segregation of hypostatic colour genes within inbred lines of chicken. *Poult. Sci.* **35**, 504–515.

DEOL, M. S., GRÜNEBERG, H., SEARLE, A. G. & TRUSLOVE, G. M. (1957). Genetical differentiation involving morphological characters in an inbred strain of mice. I. A British branch of the C57BL strain. *J. Morph.* **100**, 345–375.

GILMOUR, D. G. (1959). Segregation of genes determining red cell antigens at high levels of inbreeding in chickens. *Genetics*, **44**, 14–33.

LOEB, L., KING, H. D. & BLUMENTHAL, H. T. (1943). Transplantation and individuality differentials in inbred strains of rats. *Biol. Bull., Wood's Hole*, **84**, 1–112.

SHULTZ, F. T. & BRILES, W. E. (1953). The adaptive value of blood group genes in chickens. *Genetics*, **38**, 34–50.

Genet. Res., Camb. (1960), **1**, *pp.* 59–61
Printed in Great Britain

Mutation induced in germ cells of the foetal female mouse

By T. C. CARTER

Medical Research Council's Radiobiological Research Unit, Harwell, Berkshire, England

(*Received* 6 *June* 1959)

INTRODUCTION

In Great Britain by far the greater part of the genetically effective dose of man-made radiation to the human population is due to high-intensity irradiation of the gonads in medical radiology; about a third of it is received by post-natal males, a third by post-natal females, and a third by foetuses, mainly in the later stages of gestation (Osborn & Smith, 1956).

When estimating the genetically effective dose it has usually been assumed that all human germ cells, at whatever stage of development, are equally sensitive to the mutagenic action of radiation, but this assumption is unsupported by data. In their absence it has been thought desirable to obtain data from some other mammal; and for this reason several studies have been made of induced mutation in the mouse, using the specific-locus method. The earlier studies were all of mutation induced in spermatogonia of adult males (Russell, 1951; Russell, Russell & Kelly, 1958; Carter, Lyon & Phillips, 1956, 1958). Recently, however, this work has been extended to cover mutation induced in spermatogonia of the foetal male (Carter, 1958), in postspermatogonial cells of the adult male (Russell, Bangham & Gower, 1958) and in oöcytes of the adult female (Carter, 1958; Russell, Russell, Gower & Maddux, 1958; Russell, Russell & Cupp, 1959). The main conclusions that can be reached are:

(i) *Radiation intensity.* When spermatogonia of the adult male or oöcytes of the adult female are irradiated, the yield of mutation per rad is dependent on the intensity of the radiation, being higher when the dose is administered at a high intensity. This effect was not found in postspermatogonial cells. Other germ-cell stages have not yet been tested.

(ii) *Germ-cell stage.* With high-intensity exposures, (*a*) spermatogonia of the adult male and oöcytes of the adult female show similar induced mutation rates, (*b*) postspermatogonial cells show a higher rate, and (*c*) spermatogonia of the foetal male show a lower rate. For low-intensity exposures, (*a*) and (*b*) are true, but (*c*) has not yet been tested.

In none of this work was the mutability of the germ cells of foetal females studied; the experiment now reported partly fills this gap.

MATERIAL AND METHODS

Strain C3H females 12½ days pregnant by Strain 101 males were placed in the low-intensity γ-radiation field from a cobalt-60 source and irradiated for six successive 16-hour nights, in the course of which their foetuses accumulated a whole-body dose of 300 rad. In female mouse foetuses of up to about 14 days' gestation the germ cells are present as oögonia, thereafter entering meiotic prophase and reaching the pachytene stage at about the time of birth, after 19 or 20 days' gestation (Snell, 1941). The irradiated germ cells were therefore oögonia and early oöcytes. After irradiation the foetuses were brought to term, and 6 weeks later they were mated to males homozygous for the recessive genes *a*, *b*, c^{ch}, *d*, *se*, *p* and *s*. They were allowed to breed for up to 7 months. The progeny were scanned for the mutant phenotype associated with the seven marked loci; mutants were tested genetically.

RESULTS AND CONCLUSIONS

The data are shown in Table 1, which also summarizes earlier data on specific-locus mutation in the female mouse. Four mutations were found in the new experiment. The observed mutation rate was higher, by a factor of 3, than that

Table 1. *Mutation at seven specific loci in germ cells of the female mouse*

Age of mouse irradiated	Radiation			Progeny examined	Mutations at loci	Source of data*
	Type	Intensity	Dose		*a b c d se p s*	
Adult	γ, Co-60	50 rad/16 hr.	600 rad	10,117	– – – 1 – – –	(a)
Adult	γ, Cs–137	86 r/week	258 r	26,468	– 1 – 1 – –	(b)
—	—	—	0 r	40,918	– – – – – – –	(b)
Adult	X, 250 Kv.	96 r/min.	400 r	1,729	– – – – – – 1	(b)
—	—	—	0 r	5,845	– – – – – – –	(b)
Adult	X, 250 Kv.	92 r/min.	400 r	6,130	– 2 – 1 1 2	(b)
Foetus	γ, Co-60	50 rad/16 hr.	300 rad	18,753	– 1 2 1 – – –	(c)

* Sources of data: (a) Carter, 1958; (b) Russell, Russell & Cupp, 1959; (c) Carter, this paper.

Table 2. *Comparison of induced mutation rate in the foetal female with those in the adult*

Mutations observed in the new experiment	4
Mutations expected if adult females had been exposed:	
To acute X-irradiation	12·5
To chronic γ-irradiation	1·3

found when oöcytes of the adult female were chronically γ-irradiated, and lower by a factor of 3 than that found when they were acutely X-irradiated (Table 2). In each case the difference approaches, but does not attain, statistical significance.

It is clear, however, that the germ cells of the foetal female mouse, irradiated under the conditions of the experiment, are not grossly more sensitive than those of the adult.

I am indebted to Mr M. J. Corp for making the dose measurements; and to Miss A. Constantine, Miss D. M. Knight and Miss H. Whitehead for technical assistance.

REFERENCES

CARTER, T. C. (1958). Radiation-induced gene mutation in adult female and foetal male mice. *Brit. J. Radiol.* **31**, 407–411.

CARTER, T. C., LYON, M. F. & PHILLIPS, R. J. S. (1956). Induction of mutation in mice by chronic gamma irradiation: interim report. *Brit. J. Radiol.* **29**, 106–108.

CARTER, T. C., LYON, M. F. & PHILLIPS, R. J. S. (1958). Genetic hazard of ionizing radiations. *Nature, Lond.*, **182**, 409.

OSBORN, S. B. & SMITH, E. E. (1956). The genetically significant radiation dose from the diagnostic use of X rays in England and Wales. *Lancet*, **i**, 949–953.

RUSSELL, W. L. (1951). X-ray-induced mutations in mice. *Cold Spr. Harb. Symp. quant. Biol.* **16**, 327–336.

RUSSELL, W. L., BANGHAM, J. W. & GOWER, J. S. (1958). Comparison between mutations induced in spermatogonial and postspermatogonial stages in the mouse. (Abstract.) *Proc. Tenth Int. Congr. Genet.* **2**, 245–6.

RUSSELL, W. L., RUSSELL, L. B. & CUPP, M. B. (1959). Dependence of mutation frequency on radiation dose rate in female mice. *Proc. Nat. Acad. Sci., Wash.*, **45**, 18–23.

RUSSELL, W. L., RUSSELL, L. B., GOWER, J. S. & MADDUX, S. C. (1958). Radiation-induced mutation rates in female mice. *Proc. Nat. Acad. Sci., Wash.*, **44**, 901–905.

RUSSELL, W. L., RUSSELL, L. B. & KELLY, E. M. (1958). Radiation dose rate and mutation frequency. *Science*, **128**, 1546–1550.

SNELL, G. D. (1941). *Biology of the Laboratory Mouse*. Philadelphia: Blakiston Company.

Genet. Res., Camb. (1960), **1**, *pp.* 62–68
Printed in Great Britain

Antigenic polymorphism in a wild population of *Paramecium aurelia*

By C. R. PRINGLE
Department of Natural History, Aberdeen University
and G. H. BEALE
Department of Animal Genetics, Edinburgh University

(*Received* 17 *June* 1959)

1. INTRODUCTION

Antigenic polymorphism, as is well known, occurs in a wide variety of organisms, from bacteria to man, and *Paramecium* is no exception in this respect. Previous work (Beale, 1954; Pringle, 1956) has shown that a population of *P. aurelia* in a single isolated pond may contain individuals capable of forming a range of different types of immobilization antigen, and the range of antigens formed by a clone derived from one individual may differ from the range formed by another. Usually several representatives of alleles at each of several loci can be shown to be present in one population, if sufficient samples are taken. The mechanism whereby genes at different loci are brought to expression under control of the cytoplasm has been described in detail elsewhere (see Beale, 1957, for earlier references).

The evolutionary significance of this kind of polymorphism is by no means clear. It is possible, as often assumed, that heterozygotes containing two antigen-determining alleles have a selective advantage over the two corresponding homozygotes, and if true this would suggest a basis for the maintenance of two or more alleles in a population; but so far there is practically no direct evidence on this point. Alternatively, there may be no selective advantage of any particular antigen-determining gene or combination of genes and the polymorphism must be presumed to have arisen by mutation and chance survival of diverse types. The gene frequencies in the population would arrive at an equilibrium, depending on the mutation frequencies to and from the various alleles.

With the aim of collecting some basic information relevant to these questions, we have made a study of a single self-contained population of *P. aurelia* in Blackford Pond, Edinburgh, over a period of 6 years. Each year samples have been taken and the frequencies of various antigen-determining alleles determined.

2. MATERIAL AND METHODS

Before describing details of the actual methods, we give the following information on the biology of the organism (for further details, see Beale, 1954). Individuals of *P. aurelia* may be considered as diploid organisms, in that a single individual can contain at the most two allelic forms of a given gene. The macronucleus, however, contains many diploid sets of chromosomes. Reproduction is normally

by asexual fission, which may occur once (or slightly more) in 24 hours at 19° C., assuming excess food, but under the conditions of Blackford Pond, Edinburgh, the temperature is usually lower than this and the amount of food usually so small that the fission rate would be expected to be much less than once in 24 hours. Two kinds of sexual process occur: conjugation and autogamy. Conjugation takes place between two individuals of opposite mating type, and may or may not involve the union of diverse genotypes. During conjugation meiosis (and hence Mendelian segregation) occurs. The two ex-conjugants from a single pair are, however, genically identical, and contain one haploid set of genes from each conjugant. Autogamy involves meiosis and an internal fusion of two genically identical haploid nuclei. Thus a population of paramecia passing through autogamy may show Mendelian segregation, since a given clone may give rise after autogamy to diverse ex-autogamous animals, but all ex-autogamous clones are homozygous in respect of all their genes. Under laboratory conditions autogamy occurs at intervals of approximately sixty fissions in the particular stocks used in these experiments, with suitable nutrition, i.e. there is an interval of about sixty fissions between one autogamy and the next, or between conjugation and autogamy. Conjugation, however, can occur much more often, provided that the two opposite mating types are present and the cultures are dense enough for the animals to come into contact.

Nothing is known with certainty concerning the means whereby individuals of *P. aurelia* pass from one pond to another. Drought-resistant stages such as cysts are unknown, and it must therefore be assumed that such movement can only occur through chance carriage by birds, etc.

All the material to be described is classified according to the Sonneborn (1957) terminology as syngen 9. This term was introduced by Sonneborn in place of the older 'variety' to denote a group of individuals capable of conjugating *inter se*, of exchanging genes and giving rise to a substantial proportion of viable progeny. Syngen 9 is the predominant syngen of *P. aurelia* in Blackford Pond. A minority of individuals or this species belong to syngen 2, and in addition there are numerous specimens of other species, such as *P. caudatum* and *P. bursaria* (Pringle, 1956), in the pond.

Abundance of the material varied greatly at different times of the year. Collections were made between May and January, most abundantly in October and November. No animals were found in February, March or April. Presumably during the latter period nutritional conditions are so poor that only a very few paramecia survive.

Samples of water, together with dead leaves or other decaying vegetation, were taken into small glass vials (4 × 1 in.). After 2–4 days at room temperature the samples were examined and individual specimens of *P. aurelia* isolated into culture medium (baked lettuce infusion with *A. aerogenes*). Only a single clone of syngen 9—progeny of one wild paramecium—was taken from any one vial, thus eliminating complications due to fission in the vial of the originally collected organisms.

Table 1. *Numbers of clones containing various* G *and* X *alleles in collections made in successive years*

	G types				X types						X type unclassified
Season	G^1G^1	G^2G^2	G^1G^2	G^3G^3	X^1X^1	X^2X^2	X^3X^3	X^4X^4	X^5X^5	X^6X^6	
1953	2	0	0	0	2	0	0	0	0	0	0
1954	60	12	7	1	65	10	1	0	0	0	4
1955	91	9	2	0	82	0	0	9	7	1	3
1956	16	4	0	0	14	0	0	3	2	1	0
1957	3	0	0	0	1	0	0	2	0	0	0
1958 (E. end)	26	1	5	0	27	0	0	3	0	0	39
(W. end)	99	4	24	0	78	0	0	12	0	0	
Totals	297	30	38	1	269	10	1	29	9	2	46

Notes: (1) Previously (Pringle, 1956), the alleles were designated as follows: $G^1 = G^{510}$; $G^2 = G^{500}$; $G^3 = G^{509}$; $X^1 = X^{510}$; $X^2 = X^{530}$; $X^3 = X^{531}$; the remainder (X^4–X^6) were not recorded earlier.

(2) The season is denoted by the year in which most of the collections were made—i.e. '1954' collections were made from May 1954 till January 1955.

(3) The column 'X type unclassified' refers to cultures which died out before producing an X type antigen.

At various times after collection (sometimes as long as a year later), the G and X serotypes of every individual clone were determined by growing samples at 25° and 30° C. respectively and testing with appropriate antisera as previously described (Pringle, 1956). It should be mentioned that, in addition to the G and X types, a number of other serotypes are quite often found in syngen 9 of *P. aurelia*. When this occurs it is necessary to make repeated sub-cultures at the appropriate temperatures and where necessary screen out the unwanted types with specific antisera. Eventually every clone which did not die out during this treatment could be made to produce both G and X serotypes, i.e. there were no 'nuls' (see Beale, 1957).

3. RESULTS

The frequencies of the different G and X types are shown in Table 1. It will be noticed that heterozygotes appear amongst the G types but not amongst the X types. This is because the G types could usually be identified soon after isolation of animals from the vials, but a prolonged series of re-isolations was sometimes necessary before the X types could be obtained. During this time autogamy occurred and any heterozygotes which might have been present in the original collections would have given rise to one or other of the two possible homozygotes. In fact, X-type heterozygotes were found (e.g. X^1X^4), but to include these in Table 1 would give a misleading impression of their frequency.

For the same reason, the total numbers appearing amongst the X types are fewer than the G types, since, during the course of the long procedure described above, forty-six of the clones died out before they could be classified as regards their X type.

In Table 2 the data are combined so as to show the total numbers of individuals containing the various combinations of *G* and *X* alleles.

Table 2. *Total numbers of collected animals showing various combinations of* G *and* X *alleles*

	X^1X^1	X^4X^4	X^2X^2	X^5X^5	X^6X^6	X^3X^3	*X* alleles unclassified
G^1G^1	218	24	10	9	1	1	34
G^2G^2	26	2	0	0	1	0	1
G^1G^2	24	3	0	0	0	0	11
G^3G^3	1	0	0	0	0	0	0

4. DISCUSSION

Owing to the relatively small numbers of certain types in any given year, the data have been combined into two groups, the first for the first three seasons 1953–5, the second for 1956–8, as shown in Tables 3 and 4. Simple inspection of Table 3 is sufficient to show that there has been no change in the relative proportions of the three *G* alleles over the period of the observations. As regards the

X types, shown in Table 4, it might appear that the frequency of X^{2*} has decreased and that of X^4 has increased, but taking the data as a whole there is no significant change ($\chi^2 = 3{\cdot}7$; $P = 0{\cdot}3$, as applied to the data for X^1, X^2, X^4, X^5).

Table 3. *Total numbers of* G *alleles found over two periods each of 3 years*

	G^1	G^2	G^3
1953–1955	315	51	1
1956–1958	317	47	0

Table 4. *Total numbers of* X *alleles found over two periods each of 3 years*

	X^1	X^2	X^3	X^4	X^5	X^6
1953–1955	149	10	1	9	7	1
1956–1958	120	0	0	20	2	1

In the last year (1958), samples were collected separately from the two ends of the pond (approximately 200 m. apart). As seen in Table 1, the proportions of G and X alleles, and of heterozygotes in relation to homozygotes, are similar at opposite ends of the pond.

It is therefore concluded that there are no grounds for disbelieving that the pond contains a homogeneous population, which has been stable in genic content for 6 years.

Considering now the various combinations of G and X alleles (Table 2), it is seen that the frequencies are such as would be expected by a random combination of the various alleles at the two loci. Here it should be mentioned that the G and X genes have previously been shown by laboratory breeding experiments to segregate independently (Pringle, 1956). Assuming that on first introduction of the species into the pond, only one or a very few individuals were present, these would then be expected to multiply clonally, and if gene recombination did not occur the population would come to contain disproportionately large numbers of certain genotypes, and few or none of others. Since the data in Table 2 show no evidence of such a disproportionality, it is inferred that conjugation, gene recombination and segregation have been occurring regularly. In any case, the existence of heterozygotes is in itself proof of the occurrence of conjugation in nature. Autogamy inevitably leads to complete homozygosis; therefore conjugation must take place if any appreciable frequency of heterozygotes is to be maintained.

Turning now to the relative numbers of heterozygotes and homozygotes at the G locus, the data are summarized in Table 5. The gene frequencies are: G^1, 0·87;

* There is a slight possibility that X^2 and X^4 are identical. Animals of type X^2 had all died out before they could be compared directly with X^4, but serum prepared against X^2 type was ineffective in immobilizing X^4 animals.

G^2, 0·13; G^3, 0·004. Assuming random conjugation amongst the three types G^1G^1, G^1G^2, G^2G^2, no autogamy, and no selection of any type, the expected numbers have been calculated and are shown in Table 5. There is a large deficit of heterozygotes, which is not surprising, of course, in view of the known occurrence of autogamy. Nothing is known about the relative frequencies of conjugation and

Table 5. *Numbers of heterozygotes and homozygotes at the* G *locus*

	G^1G^1	G^2G^2	G^1G^2
Obtained	297	30	38
Calculated (see Text)	277·4	7·3	80·3

autogamy in nature, but considering the sparseness of the population opportunities for conjugation would seem to be few and one would then expect few or no individuals to be heterozygotes.

However, it must be admitted we do not know to what extent conjugation occurs under the nutritional and other conditions existing in nature, nor whether there is any significant conjugational inbreeding amongst the offspring of particular conjugating pairs. These topics are discussed at length by Sonneborn (1957).

Kimball *et al.* (1957) found in certain laboratory (test-tube) populations that conjugation was the predominant sexual process and autogamy comparatively rare, but the conditions were there highly artificial since there was an abundance of food, the cultures were dense, and meetings between animals of opposite mating types would occur frequently.

A factor which would counteract the effect of autogamy in maintaining a high proportion of homozygotes would be natural selection of heterozygotes, should such a phenomenon exist, but there is at present no information on whether such selection does in fact operate in this system. Siegel (1958) found evidence that some hybrids had a higher fission rate than pure stocks, but the comparison here involved quite distinct stocks originally isolated from widely separated natural populations, which would differ in many genes. His results are therefore not comparable with ours.

All the data on heterozygotes obtained in our experiments are concerned with the *G* locus. It is worth noting here that tests directly made on animals immediately after collection from Blackford Pond show that the G serotype is the one normally produced in nature in this population. There would thus be ample opportunity for natural selection to operate on the heterozygotes G^1G^2 containing a mixture of the two G-type antigens. With the *X* alleles, however, the situation is quite different. The antigens controlled by these genes are only formed in paramecia growing at temperatures above 28 °C., which are never reached in Blackford Pond. There would therefore be no opportunity for selection to operate on

the immobilization antigens produced by any of the *X* alleles, and it follows that only the relative frequency of conjugation and autogamy would control the proportion of heterozygotes at this locus. (This disregards the possibility that the antigen-determining genes affect, in addition to the specificity of the immobilization antigens, other unknown properties of the organisms.)

It is to be hoped that in the future estimates will be made of the proportions of heterozygotes for alleles, such as the *X* alleles, which are not expressed in nature. Assuming then an absence of selection, the discrepancy between expected and obtained proportions of heterozygotes would give an indication of the importance of autogamy. It would then be possible, by comparing such results with the proportions of heterozygotes for genes which are expressed in nature, such as the *G* alleles, to discover whether there was any effect of natural selection of the latter.

SUMMARY

1. A population of *Paramecium aurelia*, syngen 9, in Blackford Pond, Edinburgh, has been sampled over a period of 6 years and the types of immobilization antigen present identified.

2. Three antigen-determining alleles at the *G* locus and six alleles at the *X* locus were found.

3. No significant changes in relative numbers of the various alleles were apparent over the period studied, nor was there any difference between opposite ends of the pond.

4. An appreciable proportion of G-type heterozygotes was found, but the numbers were significantly less than would be expected by random mating alone. Autogamy is presumed to be responsible for this deficit.

5. Whether there is any natural selection in favour of heterozygotes over homozygotes in unknown. A possible method for obtaining information on this subject is discussed.

REFERENCES

BEALE, G. H. (1954). *The Genetics of* Paramecium aurelia. Cambridge University Press.

BEALE, G. H. (1957). The antigen system of *Paramecium aurelia*. *Int. Rev. Cytol.* **6**, 1–23.

KIMBALL, R. F., GAITHER NENITA & STELLA WILSON. (1957). Genetic studies of a laboratory population of *Paramecium aurelia*, variety 1. *Evolution*, **11**, 461–465.

PRINGLE, C. R. (1956). Antigenic variation in *Paramecium aurelia*, variety 9. *Z. indukt. Abstamm.- u. VererbLehre*, **87**, 421–430.

SIEGEL, R. W. (1958). Hybrid vigor, heterosis and evolution in *Paramecium aurelia*. *Evolution*, **12**, 402–416.

SONNEBORN, T. M. (1957). Breeding systems, reproductive methods and species problems in protozoa, in *The Species Problem*. Washington, D.C.: American Association for the Advancement of Science. Pp. 155–324.

Genet. Res., Camb. (1960), **1**, *pp.* 69–90
With 4 plates and 9 text figures
Printed in Great Britain

Two closely linked genes in the mouse

By H. GRÜNEBERG and G. M. TRUSLOVE
Medical Research Council Group for Experimental Research in Inherited Diseases, University College, London

(*Received* 1 *July* 1959)

This paper is dedicated to Professor Paula Hertwig on the occasion of her seventieth birthday

INTRODUCTION

Cases of closely linked genes with similar phenotypic effects were probably first noticed in *Drosophila melanogaster* (Grüneberg, 1937). In recent years, numerous instances of a similar kind in viruses, bacteria, fungi and *Drosophila* have served for the study of the nature of allelism and the fine structure of the gene. In mammals, the only instance of a 'complex locus' is that of the Brachyury and Anury 'alleles' (T, t^0, t^1, etc.) in the mouse which has been studied in great detail by Dunn (1956) and his colleagues.

In this paper, a new spotting gene in the mouse will be described which turns out to be closely linked to the well-known gene for dominant spotting with macrocytic anaemia (W and W^v) in linkage group III. Compared with the simple (biochemical, etc.) phenotypes of most of the microbial genes used for similar studies, the phenotypes of both of these genes in the mouse are a good deal more complicated. The detailed comparison thus made possible reveals several puzzling features for which no obvious explanation can at present be given. Presumably the complexity of the situation here encountered will not prove exceptional and will thus have to be taken into account in the formulation of a general theory of gene structure and gene action.

GENETICS

The gene to be described in this paper arose as a spontaneous mutation in the C57BL strain maintained by the Glaxo Laboratories (Greenford, Middlesex) in the year 1950. The present stock is derived from an outcross to the inbred strain CBA/Gr. 'Patch' (symbol *Ph*), to give it a name, produces spotting rather like that of piebald (s/s) and belted (bt/bt) in the mouse; i.e., areas of pigmented and of white fur are sharply demarcated from each other (Plate I) and there is little intermixture of white and pigmented hairs as in dominant spotting with macrocytic anaemia ($W/+$ and $W^v/+$). The amount of dorsal white is very variable, but there is always at least a large belly-spot together with extensive tail spotting and white digits, and the classification is thus never in doubt. No other anomalies have been discovered in the living Patch mouse.

All Patch mice tested (most of them derived from $Ph/+ \times Ph/+$ matings) have proved to be $Ph/+$ heterozygotes, and matings between such animals produce a 2 : 1 rather than a 3 : 1 ratio of spotted to self mice (Table 1). The Ph/Ph homozygote is thus inviable; as will be shown below, it dies before birth.

Table 1. *Segregation of Patch*

Type of mating	$Ph/+$	$+/+$	Total
$Ph/+ \times +/+$	192	167	359
$Ph/+ \times Ph/+$	1052	522	1574

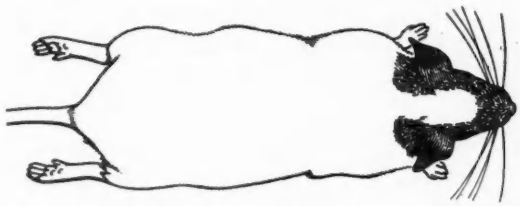
Tests were carried out to establish the relationship between Patch and some of the known major spotting genes. A cross of a $Ph/+$ to a piebald s/s mouse produced two spotted ($Ph/+$; $+/s$) and three 'self' ($+/+$; $+/s$) mice; the latter, like most $+/s$ individuals, had some white on tails and feet. One $Ph/+$; $+/s$ male was backcrossed to a $+/+$; s/s female. Ten out of the thirty-seven offspring produced resembled the black-eyed white ($W/+$; s/s) of the Mouse Fancy; being either completely white, or with small pigmented patches round the eyes, in the ear region or on the haunches. They were thus much whiter than either $Ph/+$ or s/s mice and were evidently of the constitution $Ph/+$; s/s. Sixteen backcross mice with medium degrees of spotting evidently included the genotypes $Ph/+$; $+/s$ and $+/+$; s/s; and eleven 'selfs' with tail and foot spotting and often with small belly-spots clearly represented the $+/+$; $+/s$ class. The appearance of the latter shows that *Ph* and *s* are not allelic to each other. The 10 : 16 : 11 ratio is compatible with a 1 : 2 : 1 ratio; hence the two genes are either independent of each other or, at any rate, not closely linked.

A $Ph/+$; $+/bt$ male (phenotype like $Ph/+$; $+/+$) was backcrossed to a $+/+$; bt/bt (belted) female. Six out of the twenty offspring produced were 'selfs' with white on feet and tails; one had a few white hairs on its belly. These animals are clearly $+/+$; $+/bt$, and thus prove that *Ph* and *bt* are not alleles. The remaining fourteen spotted mice included some with narrow belts (presumably $+/+$; bt/bt), some with wide belts (presumably $Ph/+$; $+/bt$) and some with head and shoulders pigmented while the rest of the body was either completely white or there was a little pigment on the haunches (presumably $Ph/+$; bt/bt). We shall give no detailed figures, as in a few animals classification by phenotype alone was not beyond doubt.

$Ph/+$; $Mi^{wh}/+$ mice resemble $Ph/+$; bt/bt animals in that pigment is confined to head, shoulders and forelegs, with sometimes a little on the haunches; the pigmented areas are dilute due to the effect of $Mi^{wh}/+$.

$Ph/+$; $W^v/+$ mice are completely white except for an area of the head which is usually like that shown in Text-fig. 1; sometimes the white extends to the tip of the nose so that only the cheeks and ears remain pigmented. The pigmented areas are diluted to a greater extent than can be accounted for by the effect of $W^v/+$ alone. Two pairs of such animals produced a small F_2 generation (Table

2), the five viable phenotypes being (1) black-eyed whites, (2) double heterozygotes as described above, (3) Patch with sharply defined areas of spotting and without coat colour dilution, (4) variegated (roan) spotting with dilution of pigment, and



Text-fig. 1. A *Ph*/+ ; W^v/+ mouse. Drawing based on photograph.

(5) self. The segregation data show that *Ph* and W^v are closely linked. Of the eighty-two gametes which went into the make-up of these forty-one F_2 mice, only two are certain recombinants; others might be hidden in class (1), but they are probably few in number.

Table 2. *Offspring of two* Ph/+ ; W^v/+ × Ph/+ ; W^v/+ *matings. Expectation on the basis of independent segregation and ignoring the inviable* Ph/Ph *homozygote.*

		Expected		
		Ratio	No. of mice	Observed
(1)	W^v/W^v; +/+ and W^v/W^v; *Ph*/+	3	10·25	18
(2)	W^v/+ ; *Ph*/+	4	13·67	21
(3)	+/+ ; *Ph*/+	2	6·83	1
(4)	W^v/+ ; +/+	2	6·83	1
(5)	+/+ ; +/+	1	3·42	0
	Total	12	41·00	41

Table 3. *Results of* $\frac{\text{Ph}+}{+\ +} \times \frac{+\ +}{+\text{W}^v}$ *matings*

$\frac{Ph+}{+W^v}$	$\frac{++}{+W^v}$	$\frac{Ph+}{++}$	$\frac{++}{++}$	Total
142	178	197	212	729

Double heterozygotes for more extensive backcross experiments were obtained from the matings summarized in Table 3. Equality of the four classes is expected. The significant deficiency of double heterozygotes indicates a reduced viability which is also obvious from their slow growth and high mortality during the first month or two. The backcross experiment (Table 4) includes a single crossover animal from a total of 1302 mice, or 0·077 per cent. The linkage between *Ph* and

W^v is thus very close. The order of the two genes in relation to a third marker is not yet known.

The F_2 and backcross linkage data are not in good agreement with each other. If in the F_2 generation there were in fact no more than the two certain crossover

Table 4. *Repulsion backcross matings* $\frac{\text{Ph}+}{+\text{W}^v} \times \frac{++}{++}$

Sex of heterozygous parent	$\frac{Ph+}{++}$	$\frac{+W^v}{++}$	$\frac{++}{++}$	$\frac{Ph\,W^v}{++}$	Total
♂	402	399	1	0	802
♀	243	257	0	0	500
Total	645	656	1	0	1302

gametes out of eighty-two (which is a likely assumption), the probability that the difference between F_2 and backcross is due to chance alone is 0·010 (Fisher's 'exact' method). Heterogeneity of linkage data is a common experience.

As with other spotting genes, the size of the white areas of *Ph*/+ mice depends on the genetic background. In outcrosses to three inbred strains (Table 5), the F_1 *Ph*/+ mice showed widely different amounts of dorsal white.

Table 5. *White areas of* Ph/+ *in three outcrosses*

Outcrossed to	Dorsal white			Belly-spot only
	Extensive	Moderate	Slight	
A/Gr	9	1	0	0
C57BL/Gr	4	5	10	0
CBA/Gr	0	0	3	11

A rather more detailed analysis of the genetic background within the Patch stock was based on the following arbitrary classes:

(1) medium belly-spot;
(2) large belly-spot;
(3) large belly-spot reaching up the flanks to form an incomplete belt (*c* and *c'*, Plate I);
(4) the same, but with complete belt (*a*, *a'* and *b*, *b'*, Plate I);
(5) as in (4), but white area incorporating one hind-leg;
(6) white area incorporates both hind-legs, but leaves pigmented areas on the haunches;
(7) haunches and forelegs also more or less completely white; pigment on head and shoulders only.

Wide belts sometimes enclose a pigmented island. Head-dots, so common in other types of spotting, have been completely absent. The whole pattern of spotting of

Ph/+ mice is best summarized as the gradual spread of a belly-spot to form a belt (sometimes with a secondary belt in the shoulder region) which then expands first posteriorly and then anteriorly. Assigning arbitrary numerical values from 1 to 7 to the seven classes, the data of Table 6 give a parent-offspring correlation of $r = 0{\cdot}70 \pm 0{\cdot}032$ (regression coefficient 0·86). This is clear evidence that the extent of white spotting in *Ph*/+ mice is largely under genetic control.

Table 6. *Correlation between parents and offspring as regards the extent of white spotting in* Ph/+ *mice*

	Midparental values											
Offspring	1	1½	2	2½	3	3½	4	4½	5	5½	6	Total
1	9	1	18	4	2				1			35
2		1	19	2	8	5		1		1		37
3	1		14	1	26	6	8	7	2			65
4			3		5	6	13	6	14	1		48
5					3	8	5	8	19	1	1	45
6					4	5	1	8		1		19
7							1				1	2
Total	10	2	54	7	48	30	28	30	36	4	2	251

A skeletal effect of Ph/+

A search for anomalies of the internal anatomy of *Ph*/+ mice has been confined to the skeleton. Papain maceration preparations of the following bones from twenty *Ph*/+ and twenty +/+ litter-mates have been examined: the skull and vertebral column, the girdles and the long limb bones and the sternebrae. The only anomaly discovered is that the interfrontal bone (Truslove, 1952) is often exceptionally large (Text-fig. 2; Table 7). This is probably largely due to an increase in the width of the skull in this region. As shown in Table 8, both

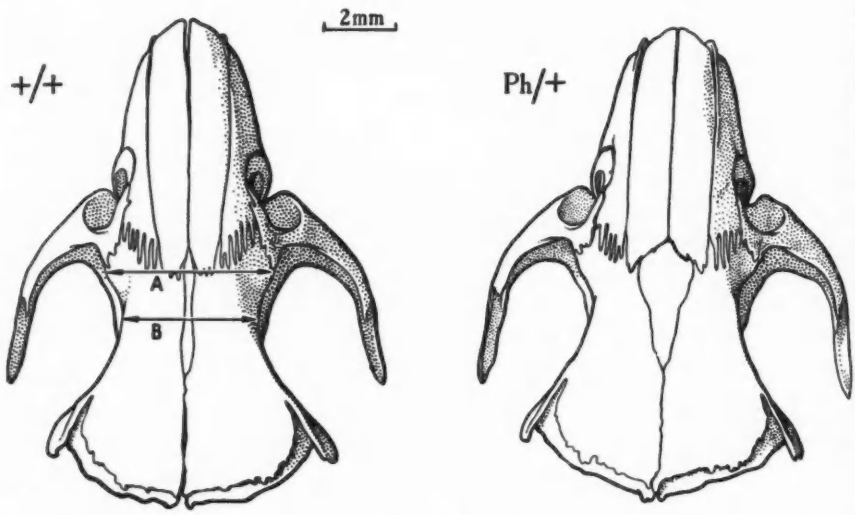
Table 7. *Size of interfrontal. Arbitrary classes.* (+) *indicates an interfrontal visible from the inside, but not from the dorsal surface of the skull*

	−	(+)	+	++	+++	++++
+/+	3	3	1	12	1	0
Ph/+	0	1	0	4	5	10

Table 8. *Skull measurements, in mm., of* Ph/+ *and* +/+ *mice*

Measurement	+/+	*Ph*/+	*t*	d.f.	*P*
A	4·81	5·01	2·85	19	~0·01
B	3·85	4·05	2·91	19	~0·01
C	15·14	14·83	3·04	19	~0·01

transverse measurements *A* and *B* are significantly greater in *Ph*/+ than in +/+ mice; by contrast, the longitudinal measurement *C* is somewhat shorter in the *Ph*/+ skulls.



Text-fig. 2. Anterior part of the skull of a normal and a *Ph*/+ litter-mate. The normal mouse has a fairly large interfrontal (++) and behind it a mild expression of parted frontals. *A* and *B* in the normal skull indicate the position of the measurements in Table 8; the longitudinal measurement *C* of that table is from the posterior margin of the basisphenoid to the nasal spine. Camera lucida drawings.

The Ph/Ph *homozygote*

Late embryos. For the identification of the lethal *Ph*/*Ph* homozygote, litters of embryos were obtained from F_2 and from backcross matings (Table 9); in the latter, the +/+ and *Ph*/+ mice came from the same families. In the F_2 matings, but not in the backcrosses, a curious monstrosity (Plates II and III) was found which is quite unlike any named entity in teratology known to us; from the most obvious feature, the name cleft-face may be suggested. These cleft-face monsters are confined to the F_2 matings and hence are obviously *Ph*/*Ph* homozygotes. But as there are only 29 cleft-face embryos out of 336 living implantations, or 8·6%, they cannot represent the whole of the *Ph*/*Ph* class. However, the F_2 matings include significantly more deciduomata (dead implantations or 'solid moles') than the backcrosses. In the F_2 matings, cleft-face embryos and deciduomata together account for 235 out of 546 zygotes, or 43·0%. Subtracting from this the 14·9% deciduomata found in the backcrosses (which do not include any *Ph*/*Ph* zygotes and hence represent the basic embryonic mortality of the stock), the remainder (28·1%) is in reasonable agreement with the 25% expectation for *Ph*/*Ph*. Hence it appears that about two-thirds of the *Ph*/*Ph* embryos die before the 12-day

Table 9. *Embryos from* Ph/+ × Ph/+ (*A*) *and from* Ph/+ × +/+ *matings* (*B*)

	Age (days)	Litters	Normal	Cleft face	Deciduomata	Dead embryos	Total	Total zygotes per litter	Viable embryos per litter
A	12	4	21	4	8	1	34	8·50	5·25
	13	44	207	18	134	2	361	8·20	4·70
	15–17	18	79	7	64	1	151	8·39	4·39
	Total	66	307	29	206	4	546	8·27	4·67
B	12	1	9*	—	—	—	9	9·00	8·00
	13	56	369	—	65	2	436	7·79	6·59
	15–18	9	59	—	12	2	73	8·11	6·56
	Total	66	437*	—	77	4	518	7·85	6·62

* Including one pseudencephalic embryo.

stage and appear only as deciduomata; the rest live to reach the cleft-face stage. Direct proof for this deduction will be given below.

Many 'normals', from both types of mating in Table 9, show a small bleb filled with a clear liquid on their foreheads; it is usually smaller than the one shown in Fig. 5, Plate II, and thus occurs roughly in the same position as the large bleb in *Ph*/*Ph* embryos. The blebs are transitory and (with a single exception) confined to 12–13-day-old embryos. Out of 772 embryos of that age from thirteen other genetically mixed stocks, animals with similar blebs were found only twice. As such face-blebs occurred both in F_2 and in backcross matings (Table 10), they cannot bear any relation to the *Ph*/*Ph* genotype; but they might be a heterozygous manifestation of *Ph*. However, this hypothesis is decisively contradicted by the

Table 10. *Face-blebs in 12–13-day-old embryos. Same material as in Table 9, but excluding forty-five embryos which were discarded prior to the discovery of this feature.*

Type of mating	Normal	Face-bleb	Total	Face-bleb %
Ph/+ × *Ph*/+	192	36	228	15·8
Ph/+ × +/+	265	67	332	20·2
Total	457	103	560	18·4

data of Table 10. There are 228 F_2 embryos, of which two-thirds, or 152, are expected to be *Ph*/+; similarly, there are 332 backcross embryos, of which one-half, or 166, are expected to be *Ph*/+. If face-bleb is a manifestation of the *Ph*/+ genotype, it should occur with the same frequency in *Ph*/+ embryos of both types of mating. The observed figures (36/152, or 23·7%, versus 67/166, or 40·4%) differ highly significantly from this expectation ($\chi^2 = 10{\cdot}08$; $n = 1$; $P = 0{\cdot}0015$). Evidently, then, face-bleb is a genetic entity whose presence in this stock is coincidental. Its distribution in the stock is not homogeneous; for instance, one family group which contributed 33 viable embryos to the F_2 and 36 to the backcross lacked face-blebs altogether; another group contributed 11 normals and 6 face-blebs to the F_2 and 45 normals and 22 face-blebs to the backcross. We reach the conclusion that face-bleb is independent of *Ph*/+ with some regret, as it might have explained an otherwise somewhat enigmatic property of *Ph*/+, the increased size of the interfrontal.

The cleft-face embryos are very uniform in appearance. The two halves of the nose are widely separated from each other by a cleft which is occupied by a large bleb or blister filled with a clear fluid (Fig. 4, Plate II; Fig. 6, Plate III). Similar subepidermal blebs occur in many other positions: in the face, on the fore- and hind-limbs, the nape of the neck, the shoulder region, the haunches and the thighs. Large paired blebs generally flank the neural tube down to the root of the tail (Fig. 7, Plate III ; Text-fig. 5); there are no blebs on the tail. The bleb fluid is clear at first, but in older foetuses (16–17 days) it tends to become haemorrhagic and such animals generally look puffed up or oedematous, particularly in the neck region. Anteriorly the neural tube is wavy and a small hole in the roof of the myelencephalon is sometimes encountered. Often the heart and parts of liver and gut are not enclosed in the body wall (ectopia cordis); this was found in 5 out of 18 embryos in one sample. The eyes may bulge considerably (Figs. 6 and 7, Plate III). Presumably as the result of the facial cleft, 15–17-day-old foetuses have a cleft palate (Fig. 6, Plate III). Two *Ph*/*Ph* embryos had a large pollex, once on the right and once on the left (Fig. 8, Plate III); normally the pollex of the mouse is rudimentary; a similar restitution of the pollex sometimes occurs in the mutants

Tail-short (*Ts*/+ ; Morgan, 1950) and luxoid (*lu*/*lu*; Forsthoefel, 1958). Cleft-face embryos progressively fall behind their normal sibs in general growth (Table 11) and apparently always die before birth.

Table 11. *Crown-rump length, in mm., of normal and cleft-face embryos. Number of individuals measured in brackets*

Age in days	Normal (N)	Cleft-face (C)	C/N
12	8·11 (20)	7·29 (4)	0·90
13	9·12 (27)	7·75 (8)	0·85
16	16·34 (16)	12·69 (4)	0·78
17	17·83 (6)	11·0 (1)	0·62

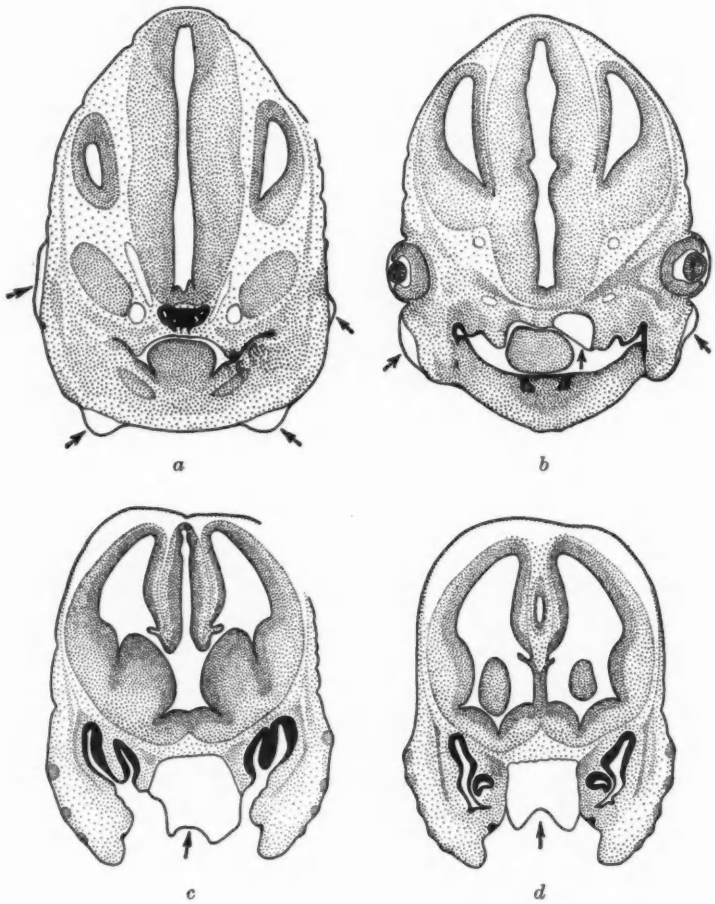
Serial sections have been prepared of two 13-day and of two 12-day cleft-face embryos (Nos. 625–626 and 642 and 644 respectively), along with four normal litter-mates (Bouin fixation, embedding by Peterfi's method; sections 10μ; Ehrlich's haematoxylin and eosin). They agree with each other in the main features and can thus be described together (Text-figs. 3–6).

The superficial blebs are situated immediately under the epidermis as in 'myelencephalic' blebs (*my*/*my*) in the mouse (Bonnevie, 1934). Some of them are small and flat and not very obvious in the intact embryo. The bleb responsible for the face-cleft is smaller in the 12- than in the 13-day embryos; it continues under the epithelium of the primitive palate (Text-figs. 3*b* and 4*c*) and comes to an end somewhat in front of the pituitary. In embryos 626 and 644 (but not in the others), a much smaller bleb occurs behind the pituitary between the epithelium of the epipharynx and the notochord (Text-fig. 4*a*); in embryo 626 it can be followed through some sixty sections (600 μ). In embryos 625, 626 and 642, anomalies occur in the cervical (and in 642 also in the thoracic and lumbar) vertebrae. The vertebral bodies are irregular in shape and tend to have bilateral double centres which sometimes are only imperfectly connected with each other. In such regions, the notochord either lies on top of the vertebral bodies (Text-fig. 5) or it is superficially embedded in them (Text-fig. 6). In embryos 626 and 642, a conspicuous and sharply defined space between neural tube and vertebral column is occupied by a tenuous web of tissue; it can be followed through many sections and its apex projects in places between the two halves of the double-centred vertebrae (Text-fig. 6). Anticipating facts to be related below, this space corresponds to a large liquid-filled cavity which occurs in younger *Ph*/*Ph* embryos; it is here evidently in the process of being filled by connective tissue.

10–11-day embryos. Six litters only are available. They are sufficient to establish the continuity between the later and the earlier manifestations of the gene, but do not justify detailed tabulation.

Ph/*Ph* embryos of this stage lack superficial blebs and the cleft face. Eleven-day F_2 litters include two kinds of abnormal embryos. In the first group are animals which are moderately retarded and have a wavy neural tube, particularly in the

cervical region; in sections there are pathological features similar to some described for the later *Ph*/*Ph* embryos. These presumably would have survived and turned into the cleft-face phenotype. The second group includes grossly retarded and highly abnormal embryos with wavy neural tubes, irregular somites and often

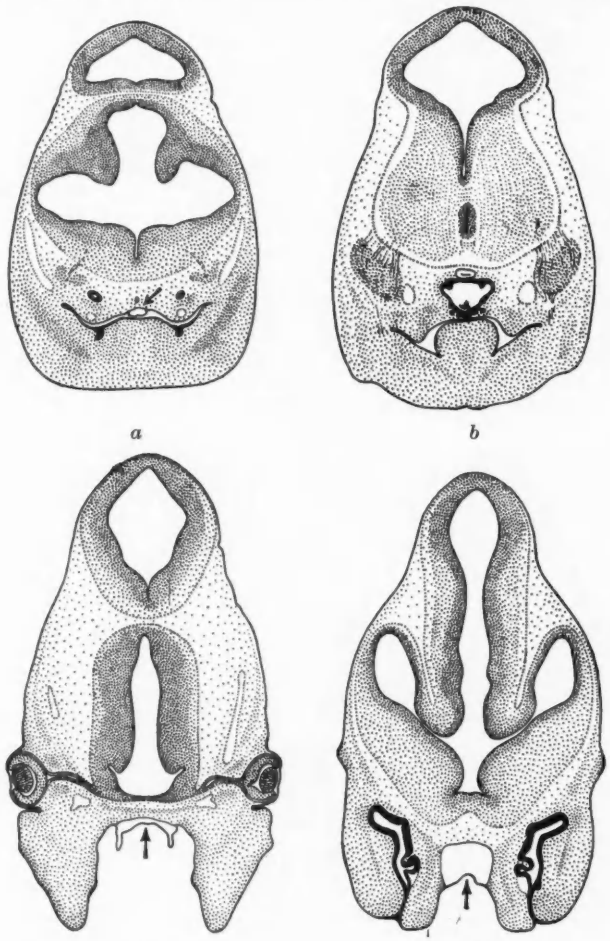


Text-fig. 3. Frontal sections through the head of a 13-day-old *Ph*/*Ph* embryo (No. 626; C.R.L. 8·4 mm.). Projection drawings made at a magnification × 35; final magnification × 17½. *a*, section through the region of the pituitary (black); *b*, through eye region; *c* and *d*, more anteriorly still. Blebs are indicated by arrows.

an enormously inflated heart and/or pericardium; they are either dead or moribund. They closely resemble certain 9-day *Ph*/*Ph* embryos and evidently represent the group which dies early.

From each of two 11-day litters, one normal, one mildly abnormal (presumed future cleft-face) and one grossly abnormal embryo were sectioned (the latter at

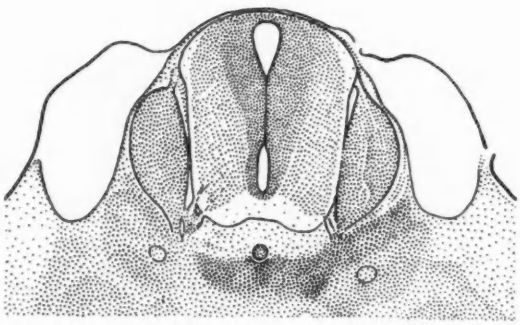
7·5μ). The two mildly abnormal embryos lack blebs under the skin. Like two of the older embryos, they have a fairly large fluid-filled cavity behind Rathke's pouch (Text-fig. 7*f*); this contains the notochord in its roof anteriorly (*e* and *f*),



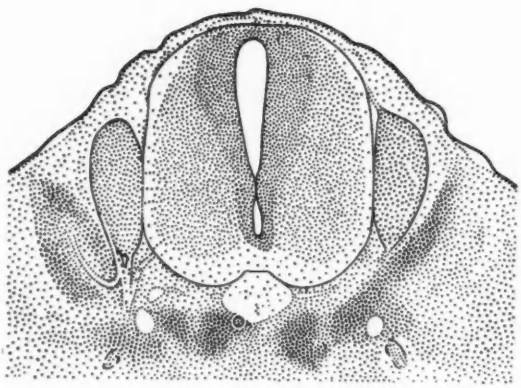
Text-fig. 4. Sections through the head of a 12-day-old *Ph*/*Ph* embryo (No. 644; C.R.L. 7·4 mm.). Magnification as in Text-fig. 3. Plane of sections about half-way between transverse and frontal. A small bleb under the epipharyngeal lining is seen in *a*; anterior to the pituitary (black in *b*), the large bleb responsible for the face-cleft is seen in *c* and *d*. A small fluid-filled space is present on either side medial to the nasal cavity and underneath the evagination of Jacobson's organ in *d*.

whereas more caudally the notochord lies dorsal to the cavity (*b*, *c*, *d*). Behind the cavity there is a roughly cylindrical space between neural tube and vertebral column with beginning ingrowth of connective tissue in one embryo, while in the other the cavity is already filled by tenuous mesenchyme.

In embryo No. 674 a bleb has just started to form in the wide concavity between the nasal pits; evidently, the epithelium can be lifted to form a blister most easily where it covers a concave surface. Once formed, the bleb becomes a mechanical hindrance to the movement of the two halves of the nose towards the midline; in



Text-fig. 5. Transverse section through the cervical region of a 13-day-old *Ph*/*Ph* embryo (No. 626; C.R.L. 8·4 mm.). Projection drawing made at a magnification × 90; final magnification × 60. Large subepidermal blebs flank the neural tube and spinal ganglia on either side, and the notochord lies dorsal to the body of the vertebra.

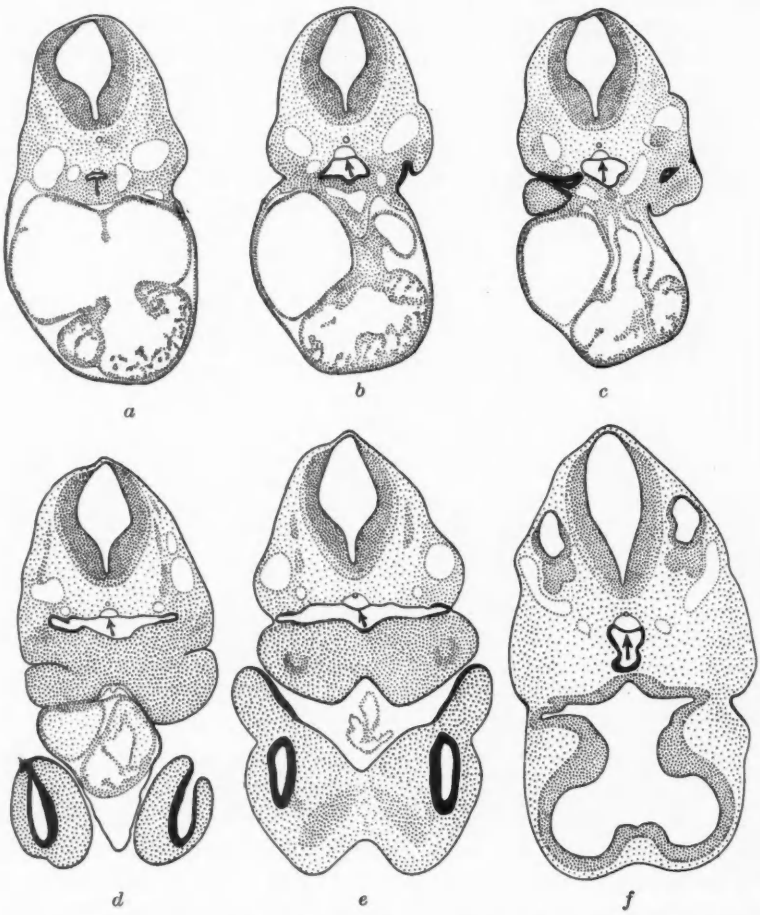


Text-fig. 6. Transverse section through the cervical region of a 12-day-old *Ph*/*Ph* embryo (No. 642; C.R.L. 7·3 mm.). Magnification as in Text-fig. 5. The vertebral body is bilateral and the notochord is superficially embedded in the left half. A sharply defined cavity between the neural tube and the vertebral column is in the process of being filled by tenuous connective tissue.

the normal embryo, this movement has already made some progress. The cleft-face phenotype found later on thus results from the prevention of movement towards the midline, not from the secondary separation of structures which had already approached one another.

Of the two highly abnormal embryos, one is certainly dead and disintegrating,

the other probably so, with many pyknotic granules in the nervous system and elsewhere. Both are enormously blown-up with liquid. In particular the heart and great vessels are dilated to many times their normal volume; it is impossible to tell



Text-fig. 7. Transverse sections through head and heart region of an 11-day-old *Ph*/*Ph* embryo (No. 672). Projection drawings made at magnification × 50; final magnification × 25. *f* passes close to the eye region, through Rathke's pouch (black) and through the ear vesicles flanking the myelencephalon; *d* and *e* pass through the branchial arch region and *e* just opens the pericardium; *a*, *b* and *c* pass through more caudal parts of the heart region. Bleb indicated by arrows.

whether the blood cells are scanty by dilution only, or whether they are also absolutely reduced in numbers. The ventricular system of the brain is dilated to a lesser extent and its wall is attenuated. Waterlogging is also encountered within the tissues, and anteriorly the notochord floats by itself in a large accumulation of liquid between the brain and the foregut. In view of the dead or moribund

condition of these embryos, one might be tempted to dismiss this fantastic 'internal drowning' as an artifact. However, very similar situations, though not quite so extreme, are encountered in 9-day *Ph/Ph* embryos which are undoubtedly alive.

9-day embryos. Eight out of eighteen F_2 litters of this stage (Table 12) were collected in 1952; the number of deciduomata in this group (16·8%) is roughly the same as the background mortality in Table 9 (14·9%). The rest of the material was collected in 1956 when the stock happened to be fairly inbred, and, as a consequence, had a much higher embryonic mortality; this is, however, virtually the same in the F_2 and backcross data (38·1 and 39·8% respectively); hence, at the 9-day stage the *Ph/Ph* embryos are still alive. This is borne out by direct observation.

Table 12. *9-day embryos from* Ph/+ × Ph/+ (*A*) *and from* Ph/+ × +/+ *matings* (*B*)

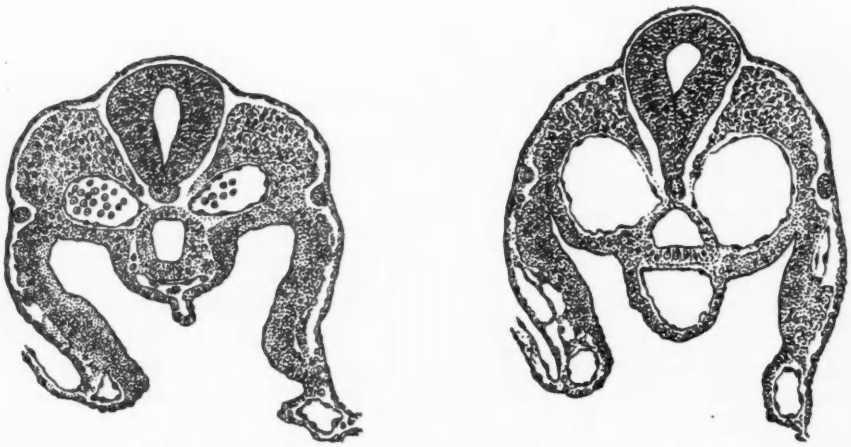
	Year	Litters	Normal	*Ph/Ph*	?	Total living embryos	Deciduomata	Grand total	Deciduomata %
A	1952	8	39	12	3	54	11	65	16·8
	1956	10	46	8	6	60	37	97	38·1
	Total	18	85	20	9	114	48	162	—
B	1956	14	71	—	–	71	47	118	39·8

Nearly all the abnormal (*Ph/Ph*) embryos of this stage can be distinguished from their normal litter-mates by external inspection. They have wavy neural tubes similar to those found in later stages. More severely affected individuals have irregularities of the somites, and the most abnormal ones are inflated with enormous hearts, or hearts of about normal size inside a huge pericardium. Depending on the degree of abnormality, *Ph/Ph* embryos are retarded to a greater or lesser extent as compared with their normal litter-mates. The typical normal 9-day embryo is U-shaped and has just completed its turning movement. Corresponding *Ph/Ph* embryos have often completed the turning movement at both ends of the body, but not yet in the middle, which is thus still more or less widely open; evidently, this delay in the closure of the ventral body wall together with an enlargement of heart or pericardium is the cause of the ectopia cordis mentioned in an earlier section. In a few younger litters of this group, in which the normal embryos have not yet completed their turning movement, *Ph/Ph* embryos are less easy to recognize by external inspection, and classification must rely mainly or entirely on the sectioned material.

All 9-day F_2 embryos have been serially sectioned (at 7·5μ) except four normals and one *Ph/Ph* embryo from a litter in the 1952 group. Twenty out of 105 fully classified embryos have been identified as *Ph/Ph* homozygotes, in reasonable agreement with the 3 : 1 expectation ($\chi^2 = 1{\cdot}98$; $n = 1$; $P = 0{\cdot}16$ approx.). Five out of nine unclassified embryos were damaged, and another specimen was a very retarded (pre-somite) embryo. Two of the three remaining doubtful specimens

have been classified as 'probably *Ph*/*Ph*'. The agreement with the Mendelian expectation is thus quite satisfactory. No abnormal embryos resembling the *Ph*/*Ph* class have been observed in the backcross matings. It has not been deemed necessary to section these seventy-one embryos.

All 9-day *Ph*/*Ph* embryos examined had accumulations of a clear liquid flanking the notochord (compare Figs. 9 and 10 with 11 and 12 and with 13 and 14, Plate IV). In the older embryos of this group, the liquid is more often found in the anterior parts of the trunk up to, and including, the region of the myelencephalon; in younger ones it tends to be more marked posteriorly. The amount of liquid is variable, but, almost without exception, *Ph*/*Ph* embryos can be distinguished from their normal sibs by this feature alone. In some *Ph*/*Ph* embryos, the heart and the whole vascular system is dilated (Text-fig. 8), sometimes to an extraordinary

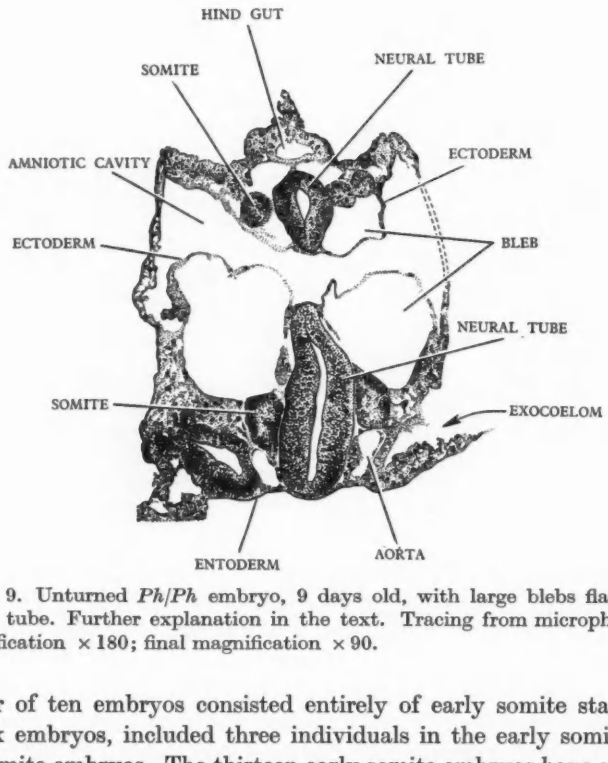


Text-fig. 8. Transverse sections through the trunk region of a normal and a *Ph*/*Ph* embryo (litter-mates, 9 days old). Note the dilation of the dorsal aortae and of the common umbilical artery. Projection drawings made at magnification × 200; final magnification × 100.

extent; bulging blood-vessels may leave fairly deep impressions on the surface of the neural tube and are responsible for some, at least, of the irregularities visible externally. In some cases, the circulatory system is not much dilated, but large quantities of liquid are found inside the pericardium; a rather mild case of this kind is shown in Fig. 11, Plate IV. Particularly in younger stages, i.e. before the turning movement is complete, some *Ph*/*Ph* embryos have large subepidermal blebs flanking the neural tube; in the embryo shown in Text-fig. 9, which is still completely unturned, each of the two long blebs is cut twice; liquid also penetrates between the somites, and it appears that the somite irregularities found in all more severely affected embryos must be ascribed to this fact. Subepidermal blebs in other positions are sometimes encountered in badly affected embryos, and there may be oedema of the mesenchyme.

Evidently the more severely affected *Ph/Ph* embryos die soon after the ninth day, and only the more mildly affected individuals survive to reach the cleft-face stage; these, at the 9-day stage, have some para-chordal liquid, but are otherwise comparatively normal.

8-day embryos. Even more than those of the preceding group, 8-day embryos of the present material differ considerably in the stage of development which they have reached, both within and between litters. All of them have been serially sectioned (at 7·5μ) within their membranes and within the decidua. They all come from $Ph/+ \times Ph/+$ matings.



Text-fig. 9. Unturned *Ph/Ph* embryo, 9 days old, with large blebs flanking the neural tube. Further explanation in the text. Tracing from microphotograph, magnification × 180; final magnification × 90.

One litter of ten embryos consisted entirely of early somite stages; another litter, of six embryos, included three individuals in the early somite stage and three pre-somite embryos. The thirteen early somite embryos have somite counts ranging from 3 to 10. In some of them the allantois has made contact with the ectoplacental cone, in others it has not. The nominal age of this group may be given as approximately 8½ days. We have failed to discover any abnormalities in this group which can be ascribed to the *Ph/Ph* genotype.* On a 3 : 1 expectation, the probability of finding thirteen normals and no *Ph/Ph* is 0·0238. It is thus improb-

* In two embryos, maternal blood was present in the exocoelom, the ectoplacental cavity and/or the amniotic cavity. It is virtually certain that this was due to an injury inadvertently inflicted on the mother immediately before she was killed. In order to save those females of the 8-day group which had failed to become pregnant despite their vaginal plugs, all of them

able that this sample failed to include any *Ph*/*Ph* embryos. If some such embryos were, in fact, present, they were either still phenotypically normal or, if they were not, their abnormalities were of a kind which we have failed to recognize. Needless to say, we have not discovered abnormalities in the twenty-five still earlier (pre-somite) embryos which we have examined.

The essential abnormality of the *Ph*/*Ph* embryo is an increase of its water content (hydrops). This develops between the 8- and 9-day stage. Abnormal accumulations of liquid are found flanking the notochord first posteriorly and later anteriorly. To a varying extent, excess liquid is also found in the circulation, in the pericardium, under the epidermis and within the tissues. The more extremely affected embryos die round about the tenth day. The survivors regularly develop a subepidermal bleb on the concavity of the primitive palate which becomes the mechanical cause for the facial cleft.

The more extreme manifestations of *Ph*/*Ph* show at a glance that the total water content of the embryo is greatly increased and that the excess liquid must thus have come from outside the embryo. Whether it enters the embryo via the allantois, or whether it is derived from the amniotic cavity or from the extraembryonic coelom, must remain an open question. It is, however, clear that the total quantity of excess liquid continues to increase and that the *Ph*/*Ph* embryo remains leaky for some considerable time.

DISCUSSION

The genes for *Ph* and *W* are located within 0·077 cM. of each other. What is the probability that a new spotting gene should be located so close to a known one? Casting the net very widely and including some rather doubtful entities, there are about fifteen known loci in the mouse which can be regarded as 'spotting genes'. The total map length of $\pm 0{\cdot}1$ cM. round these fifteen loci is 3·0 cM. According to Carter (1955), the total genetic map length of the mouse amounts to $16{\cdot}2 \pm 3{\cdot}5$ M. Hence, as a first approximation, the probability of a new spotting gene being located within 0·1 cM. of a known one is 3/1620, or 0·00185. Without going into refinements, it is thus clear that the close proximity of *Ph* and *W* is probably not a chance event; it thus becomes necessary to compare their effects in more detail by reference to Table 13.

The question to be answered is whether *Ph* and *W* are non-complementary (i.e. alleles, though, of course, with different mutational sites), or whether they are complementary to each other like the *Drosophila* doublets which were mentioned in the Introduction. This question is more easily asked than answered. In the case of two recessive genes, the mutant phenotype of the compound a^1/a^2 is generally taken as the criterion for functional allelism. No such criterion is at

had their uteri palpated for pregnancy just before they were killed. This practice was later discontinued when these two (and other) embryos showed the unforeseen damage thus caused.

It is very probable that similar lesions of the trophoblast in putative hydrocephalus-1 embryos which Bonnevie (1945) described as an early manifestation of that gene were, in fact, traumatic as explained above.

present available in the case of dominant genes such as *Ph* and *W*. We will now discuss each item of Table 13 in more detail.

Table 13. *The main effects of the* Ph *and* W *loci*

		Ph/+	*Ph*/*Ph*	*W*/+	*W*/*W*	W^v/+	W^v/W^v
1.	Spotting	+	?	+	+++	+	+++
2.	Dilution	(+)	?	—	—	+	—
3.	Anaemia	(+)	?	—	+++	+	++
4.	Sterility	—	—	—	+	—	+
5.	Hydrops	—	+	—	—	—	—

(1) *Spotting*. Most of the spotting of *Ph*/+ mice consists of a sharply defined ventral area with or without belt. However, some roan or variegated spotting like that of *W*/+ and W^v/+ may occur, particularly in the belt region and/or the shoulders. How much weight should be given to the interaction of the genes in the *Ph*+/+ W^v double heterozygote is somewhat problematical. Interactions between non-allelic spotting genes (as in the black-eyed white *W*/+ ; *s*/*s* mouse) have been known for a long time; and *Ph* itself also interacts rather strikingly with *s*/*s*, *bt*/*bt* and Mi^{wh}/+. But this does not exclude the possibility that the interaction with W^v might be specific.

Could the phenotype of the *Ph*+/+ W^v mouse be regarded as equivalent to that of a compound *Ph*/W^v if the two entities are alleles? W^v/W^v mice are black-eyed whites but their ears are sometimes a little sooty. *Ph*/*Ph* embryos have pigmented eyes (Plates II and III), and presumably, if they lived to grow hair, they would be whiter than the *Ph*/+ heterozygote. If they are potential black-eyed whites, the phenotype of *Ph*+/+ W^v would be partly complementary. If, on the other hand, *Ph*/*Ph* is potentially a white mouse with a pigmented head, *Ph* and W^v could be regarded as non-complementary, with *Ph* dominant over W^v. The question must remain open until the potential fur colour of various regions of *Ph*/*Ph* embryos has been established by transplantation experiments. That such experiments are practicable has been shown by Rawles (1947) in normal 8–12-day mouse embryos, and by Auerbach (1954) in the case of the Splotch (*Sp*/*Sp*) homozygote which dies at the beginning of the thirteenth day of development.

(2) *Dilution*. Dilution of the fur pigmentation in the pigmented areas occurs in W^v/+, but not in *W*/+ heterozygotes. By itself, *Ph*/+ does not dilute fur pigmentation in agouti and non-agouti (*a*/*a*) mice as judged by eye. However, the *Ph* gene seems to interact with W^v in this respect. In *Ph*+/+ W^v mice, the pigmented areas of the head are a little paler than in W^v/+ animals. It is difficult to be certain whether this is brought about by a general dilution of hair pigmentation or by an admixture of white hairs to the fur; in either case, an interaction is indicated as *Ph*/+ by itself has neither effect.

(3) *Anaemia*. As first shown by one of us (Grüneberg, 1939, 1942) and subsequently confirmed by many other authors, *W*/*W*, *W*/W^v and W^v/W^v mice suffer

from a macrocytic anaemia; in a very mild form this is also present in $W^v/+$, but not in $W/+$ mice. For purposes of comparison, we have examined 13–14-day-old $Ph/+$ mice although, in view of the haematological normality of the $W/+$ heterozygote, only positive findings would be decisive. Haemoglobin was estimated by means of the M.R.C. grey-wedge photometer; the volume of packed cells was determined with a Hawksley micro-haematocrit centrifuge; and cell counts were made in the usual way in an improved Neubauer counting chamber.

Comparing $++/++$ with $Ph+/++$ mice (Table 14), it is obvious that *Ph* by itself has no appreciable effect on the red-blood picture of 13–14-day-old mice; however, slight effects, or effects at other stages of development, are not excluded. The situation is less clear on the right side of Table 14. $Ph+/+\ W^v$ mice have

Table 14. *The red-blood picture in 13–14-day-old animals. Averages of fifteen animals in each case. Animals in the first two and in the last two columns are litter-mates. In two instances in the last two columns, two animals of either kind were taken from the same litter; in view of intra-litter correlations, the mean values of the two pairs have been used in significance tests so that there are only twelve degrees of freedom in* t *tests*

	$++/++$	$Ph+/++$	$++/+\ W^v$	$Ph+/+\ W^v$
Hb (g./100 ml.)	11·0	10·8	10·0	9·7
R.B.C. per mm^3	5,628,000	5,535,000	4,629,000	4,267,000
Haematocrit %	33·7	32·7	30·7	29·6
Mean corpuscular volume in μ^3	60·9	59·3	66·9	69·4
Mean Hb concentration %	32·6	33·0	32·5	33·0
Mean Hb content in $\gamma\gamma$	19·6	19·6	21·7	22·9

rather lower blood counts (R.B.C.) than their $++/+\ W^v$ litter-mates; the difference is, however, barely significant ($t = 1{\cdot}835$; $n = 12$; $P = 0{\cdot}09$; considering that the difference is in the expected direction, $P = 0{\cdot}045$), and the differences as regards Hb and haematocrit are not significant ($t = 1{\cdot}11$ and $1{\cdot}50$ respectively). Both $W^v/+$ genotypes show a mild macrocytic anaemia as compared with $+/+$ mice in conformity with previous findings (Grüneberg, 1942; and others). But the red cells of $Ph+/+\ W^v$ are not significantly more macrocytic than those of $++/+\ W^v$ mice ($t = 0{\cdot}94$; $n = 12$) which might have been expected in the case of a specific interaction. The increased anaemia of the double heterozygotes, if real, is thus certainly slight. The possibility must also be considered that their low cell count may be an expression of their retardation rather than its cause.

The *Ph/Ph* embryo presents almost insuperable difficulties to the application of standard haematological techniques. Bouin-fixed sectioned material stained with H. & E. is not suitable for the detection of anomalies of the red-blood picture except the very coarsest ones. Repeatedly we have thought we had discovered cytological differences in the embryonic red blood cells, but we do not regard the

question as settled either way. A fresh start with more suitable techniques will thus be required.

(4) *Sterility.* W/W, W/W^v and W^v/W^v mice are sterile or nearly so. As shown by Mintz & Russell (1957), their primordial germ cells form normally at 8 days, but subsequently they fail to multiply and are retarded in their migration from the yolk-sac to the germinal ridges. Using the same histological technique, we have examined two normal and two *Ph*/*Ph* 10-day embryos from an F_2-type litter. Normal and *Ph*/*Ph* embryos had similar numbers of primordial germ cells, and though in the absence of complete counts smaller differences cannot be excluded, there is certainly no *prima facie* case indicating sterility in the *Ph*/*Ph* mouse if it survived to breeding age.

(5) *Hydrops.* No disturbances of embryonic liquids comparable to those found in *Ph*/*Ph* homozygotes have been described for W/W or W^v/W^v embryos. Obviously, none severe enough to be lethal or to lead to cleft-face are present. If mild hydrops perhaps occurs in 9–10-day-old W/W etc. embryos, it might be detectable in the material of Mintz & Russell (1957). Disturbances of liquid balance (oedema, blebs) are often encountered in amphibian embryos which have been subjected to surgical interference; so perhaps these disturbances in *Ph*/*Ph* embryos (and those in 'myelencephalic' blebs, *my*/*my*; Bonnevie, 1934) may be comparatively unspecific and rather far removed from the primary gene action.

While, at present, it is not possible to state with confidence that the interaction between two dominant genes is specific, it may be possible to state with confidence that it is not. If, for each gene separately, developmental studies have established a pedigree of causes of the pleiotropic effects, an interaction which involves remote gene effects is clearly not specific.

Unfortunately, despite the great effort by many workers which has gone into the analysis of the *W*-series, the causal connexions between the effects on pigmentation, blood and germ cells have not yet been clarified. The same applies to the pleiotropic effects of *Ph*, as indeed to a long series of genes in the mouse which involve pigmentation along with a variety of other organ systems.

The facts that *Ph*/*Ph* mice are potentially fertile while W/W and W^v/W^v mice are sterile, and that *Ph*/*Ph* embryos are hydropic while W/W and W^v/W^v are not, cannot be evaluated until the position of these effects in the respective pedigrees of causes is known. A discrepancy of secondary effects is obviously of less weight than a discrepancy in fundamentals.

The relationship between *Ph* and the *W*-series thus remains problematical at present. The data presented are clearly not a strong case for the existence of a specific (allelic) interaction between the genes, but they do not rule it out. Perhaps the strongest indirect evidence against a specific interaction is the existence of the gene for Steel (*Sl*; Sarvella & Russell, 1956; Bennett, 1956) in the mouse. This gene is a very close mimic of W^v, both in the heterozygous and in the homozygous condition; in the *Sl*/+ mouse, there is spotting, dilution of coat colour and a mild macrocytic anaemia; *Sl*/*Sl* is potentially a black-eyed white, it has an extreme macrocytic anaemia which usually kills it before birth, and it has a clear involve-

ment of the primordial germ cells. The close similarity between *Sl* and W^v (which segregate independently of each other) is evidence for a close causal connexion between the pleiotropic effects; hence a gene which interferes with the hypothetical pedigree of causes near its root will produce the full triad. If this reasoning is valid, the marked differences between *Ph* and the *W*-series suggest that they are not alleles, but a gene doublet with complementary action.

SUMMARY

(1) In heterozygous condition, the gene for Patch (symbol *Ph*) produces spotting with sharply defined pigmented and white areas. The extent of the spotting is under the control of the genetic background.

(2) The *Ph*/*Ph* homozygote is inviable and dies before birth. In 9-day embryos, clear liquid is found flanking the notochord; in addition, there may be excessive amounts of liquid in the pericardium, the circulation, the tissues, and under the epidermis. The more extremely affected *Ph*/*Ph* embryos die at about 10 days. About one-third survive to later stages of pregnancy. Such 'cleft-face' embryos have a large bleb of liquid in the middle of the face which interferes mechanically with the formative movements of the nose and palate, and many subepidermal blebs elsewhere.

(3) *Ph* is closely linked to the gene for dominant spotting with macrocytic anaemia (*W*, W^v) in linkage group III, the crossover percentage being 0·077. There is a striking interaction in the double heterozygote *Ph*+/+ W^v which is almost white, but nearly equally striking interactions occur with other spotting genes (*s*, *bt* and Mi^{wh}).

(4) By itself, *Ph* has no detectable effect on the coat colour, but in the double heterozygote with W^v, it slightly increases the dilution effect of the latter in the regions which remain pigmented. Similarly, *Ph* by itself has no appreciable effect on the red-blood picture of 13–14-day-old animals, but in the double heterozygote with W^v, it probably slightly increases the mild macrocytic anaemia produced by that gene. It remains unknown whether the *Ph*/*Ph* homozygote has an effect on the blood. Unlike *W*/*W* and W^v/W^v, *Ph*/*Ph* has no appreciable effect on the primordial germ cells.

(5) The relationship between *Ph* and the *W*-series is discussed.

The authors are deeply indebted to Dr W. F. B. Cuthbertson (Glaxo Laboratories, Greenford, Middlesex) for the gift of the original Patch animals. Dr A. G. Searle kindly looked after the stock during the summer of 1952. The microscopic preparations were made by Miss Hermingard Bartels-Walbeck and by Miss Renate Kühne. The photographs of Plate I were taken by the late Mr A. J. E. Munday and the microphotographs of Plate IV by Miss Heide Schulze. Mr A. J. Lee prepared the drawings of Plates II and III and assisted with many of the text-figures. The work described in this paper was supported by a grant from the Rockefeller Foundation which is gratefully acknowledged.

REFERENCES

AUERBACH, R. (1954). Analysis of the developmental effects of a lethal mutation in the house mouse. *J. exp. Zool.* **127**, 305–330.

BENNETT, D. (1956). Developmental analysis of a mutation with pleiotropic effects in the mouse. *J. Morph.* **98**, 199–234.

BONNEVIE, K. (1934). Embryological analysis of gene manifestation in Little and Bagg's abnormal mouse tribe. *J. exp. Zool.* **67**, 443–520.

BONNEVIE, K. (1945). Hereditary hydrocephalus in the house mouse. III. Manifestation of the *hy*-mutation in embryos 9–11 days old, and younger. *Skr. norske VidenskAkad., I. Mat.-Naturv. Kl.*, 1944, No. 10, pp. 60.

CARTER, T. C. (1955). The estimation of total genetical map lengths from linkage test data. *J. Genet.* **53**, 21–28.

DUNN, L. C. (1956). Analysis of a complex gene in the mouse. *Cold Spr. Harb. Symp. quant. Biol.* **21**, 187–95.

FORSTHOEFEL, P. F. (1958). The skeletal effects of the luxoid gene in the mouse, including its interactions with the luxate gene. *J. Morph.* **102**, 247–287.

GRÜNEBERG, H. (1937). Gene doublets as evidence for adjacent small duplications in *Drosophila*. *Nature, Lond.*, **140**, 932.

GRÜNEBERG, H. (1939). Inherited macrocytic anemias in the house mouse. *Genetics*, **24**, 777–810.

GRÜNEBERG, H. (1942). Inherited macrocytic anaemias in the house mouse. II. Dominance relationships. *J. Genet.* **43**, 285–293.

MINTZ, B., & RUSSELL, E. S. (1957). Gene-induced embryological modifications of primordial germ cells in the mouse. *J. exp. Zool.* **134**, 207–238.

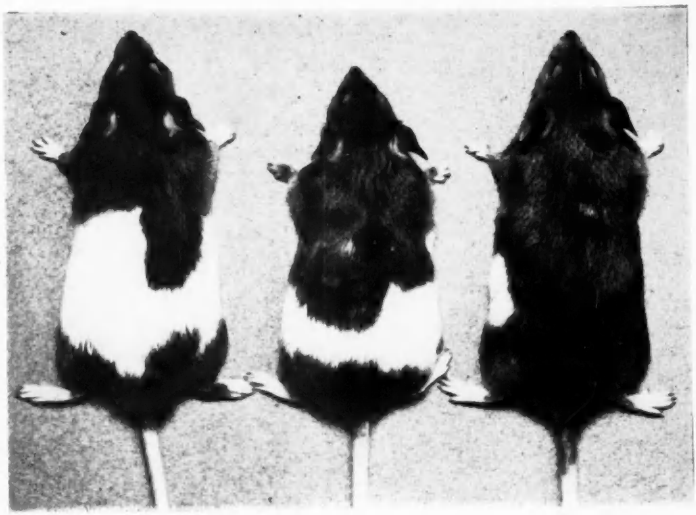
MORGAN, W. C. (1950). A new tail-short mutation in the mouse whose lethal effects are conditioned by the residual genotype. *J. Hered.*, **41**, 208–215.

RAWLES, M. E. (1947). Origin of pigment cells from the neural crest in the mouse embryo. *Physiol. Zool.*, **20**, 248–266.

SARVELLA, P. A. & RUSSELL, L. B. (1956). Steel, a new dominant gene in the house mouse with effects on coat pigment and blood. *J. Hered.* **47**, 123–8.

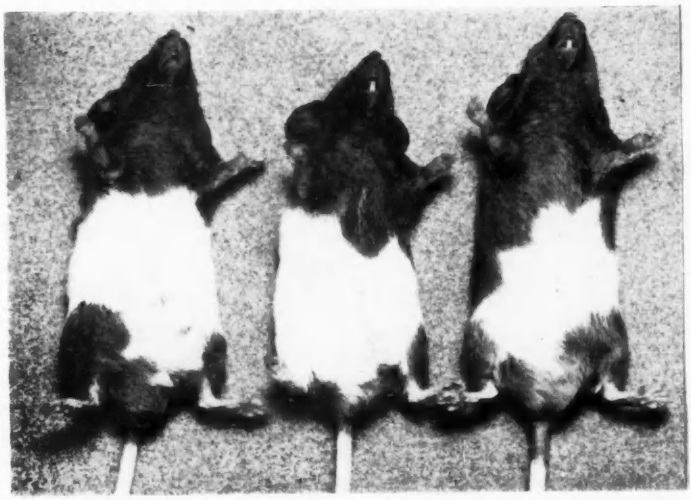
TRUSLOVE, G. M. (1952). Genetical studies on the skeleton of the mouse. V. 'Interfrontal' and 'parted frontals'. *J. Genet.*, **51**, 115–22.

1.



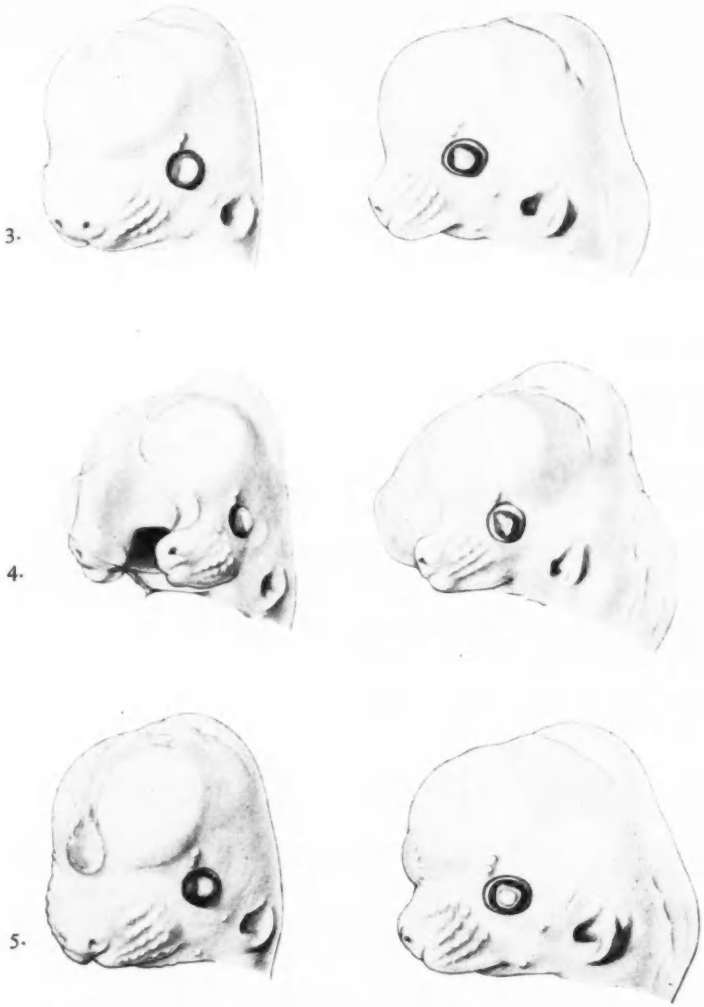
a *b* *c*

2.

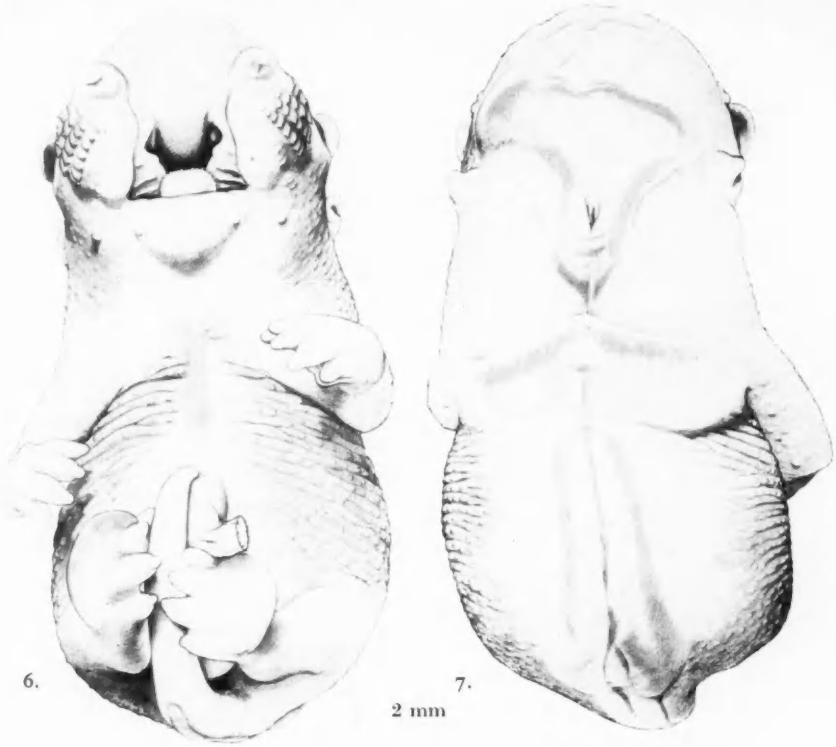


a′ *b′* *c′*

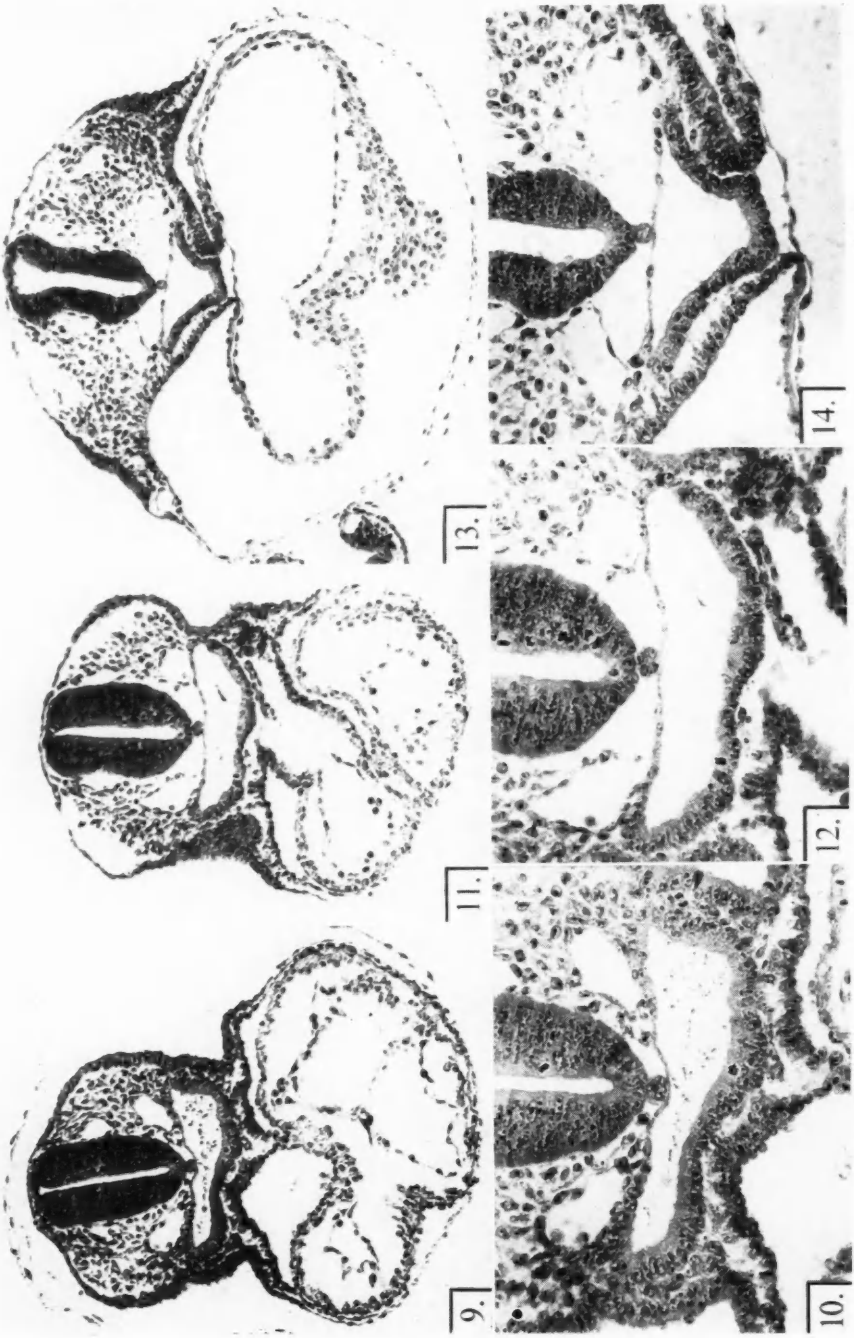
Dorsal and ventral views of three *Ph*/+ heterozygotes.



Heads of 13-day embryos. Fig. 3, normal; Fig. 4, cleft-face (*Ph*/*Ph*); and Fig. 5, normal with face bleb.



Figs. 6 and 7: ventral and dorsal views of a 16-day cleft-face (*Ph*/*Ph*) embryo. Fig. 8: the left fore-limb of the same embryo.



Transverse sections through the heart region of three 9-day embryos (litter-mates). Figs. 9 and 10 normal; Figs. 11 and 12 and Figs. 13 and 14, *Ph/Ph*, embryos. Bouin fixation, embedded by Peterfi's method, sectioned at 7·5 μ. Heidenhain's iron haematoxylin and eosin. Figs. 9, 11 and

Genet. Res., Camb. (1960), **1**, *pp.* 91–113
With 7 text figures
Printed in Great Britain

Selection of mice for growth on high and low planes of nutrition

By D. S. FALCONER
Agricultural Research Council Unit of Animal Genetics, Institute of Animal Genetics, Edinburgh, 9

(*Received* 27 *July* 1959)

INTRODUCTION

The choice of environment, when selection is to be applied for the improvement of farm animals, presents a problem that is still incompletely solved. Should the breeder subject the animals among which selection is to be made to a 'good' environment which gives maximal expression to the desired character? Or should he rather subject them to the conditions, whether good or bad, under which the improved breed is destined subsequently to live? Considerations of genotype-environment interaction, or the specialization of breeds, point to the latter course as likely to be the better, and this view can be supported by theoretical considerations of the genetic correlation between two characters (Falconer, 1952). Yet an experiment with mice (Falconer & Latyszewski, 1952) did not fully substantiate the theoretical expectations. The improvement made by selection under good conditions was not carried over when the animals were transferred to bad conditions, but the improvement made under bad conditions was retained when the animals were transferred to good conditions. Selection under bad conditions therefore produced animals that performed well under both good and bad conditions. This experiment, however, had several defects, and such unexpected results clearly needed confirmation by another experiment. The experiment described here was therefore undertaken with the object of exploring the problem afresh. It was designed so as to provide a test of the adequacy of the theory of selection from which predictions might be made of the responses to be expected when selection is made under different environmental conditions.

The theoretical considerations are based on the idea that if a character is measured under two different environmental conditions it may be treated not as one but as two different characters. For example, the growth of animals reared on a high plane of nutrition is one character and the growth of animals reared on a low plane of nutrition is another. The genetic similarity between the two characters is expressed as the genetic correlation between them. From a knowledge of the genetic correlation and of the heritability of each character it should be possible to predict the improvement to be expected in one character when selection is applied to the other; to predict, in other words, how much of the improvement made by selection in one environment will be carried over when the improved breed is transferred to another environment.

The general plan of the experiment was as follows. Two-way selection was applied to one pair of lines for growth-rate when the individuals were reared on a high plane of nutrition, and to another pair of lines for growth-rate when the individuals were reared on a low plane of nutrition, one line of each pair being selected for increased growth and the other for decreased growth. Some individuals in each generation were reared on the other nutritional plane, so that in every generation there was a measurement of the mean growth in both environments; or, in other words, a measurement of the mean of both characters. Thus a comparison of the correlated and direct responses could be made for both characters. Two estimates of the genetic correlation could then be obtained, in a manner to be explained in a later section, and if the responses were in accord with the theoretical expectations, these two estimates should be the same. This analysis of the responses in terms of the genetic correlation will be found at the end of the Results section.

EXPERIMENTAL PROCEDURE

Stock

The stock of mice used (known in the laboratory as the C-stock) was constructed in 1949 from crosses between Bateman's high-lactation line, a line selected for large size after a cross of MacArthur's with Goodale's large lines, and two stocks carrying a variety of mutant genes and having the inbred line C57BL/Fa in their ancestry. The stock was first used in another experiment (Falconer & Robertson, 1956), which, however, produced no detectable genetic change. The 'experimental' and 'control' lines of that experiment were crossed in 1952 to provide the foundation population of the present experiment. The foundation population was divided into two sets of twelve single-pair matings which constituted generation 0 of the selection programme. One set of twelve matings was the starting point of selection for one character and the other set for the other character.

Characters selected

All mice were weighed at 3 weeks and at 6 weeks of age, and selection was based on the growth during this period of 3 weeks. Selection was made both for increased growth and for decreased growth, so that the two sets of matings in generation 0 were further divided each into two lines, one selected upwards and the other downwards. The lines selected for increased growth (large lines) will be indicated by the symbol +, and those selected for decreased growth (small lines) by the symbol —. The mice of one large and one small line were reared on the standard cubed diet, which provided a high plane of nutrition. These will be referred to as 'high-plane lines', with the symbol H. The mice of the other pair of lines were reared on a specially prepared diet, to be described below, which provided a low plane of nutrition. These lines will be referred to as 'low-plane lines', with the symbol L. Thus the H-lines were selected for the character 'growth on high plane' and the L-lines were selected for the character 'growth on low plane'. These diets were administered to the first litters of all matings, and the selection

was made exclusively among mice born in these first litters. The mice of all second litters were reared on the other diet; that is to say, mice of the H-lines were reared on low plane, and mice of the L-lines on high plane. Thus, the second litters provided measurements of the correlated responses of the characters not directly selected. (An error in the routine for the exchange of diets between first and second litters was probably made at generation 11. The observed growth, seen best in Fig. 5, suggests that the second litters of both high-plane lines were

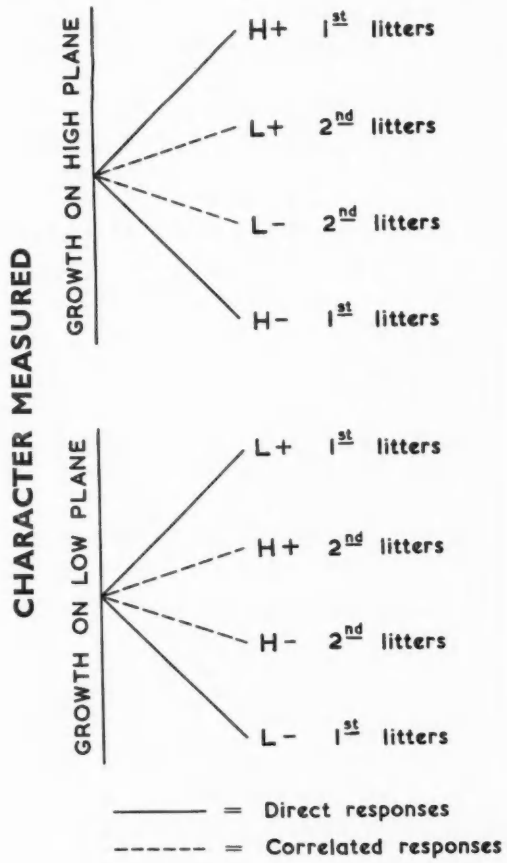


Fig. 1. Diagrammatic plan of the experiment.

reared on high plane instead of on low plane. The values are entered in the graphs as observed, but they are excluded from the computations). Table 1 summarizes the designations and treatments of the lines, and Fig. 1 summarizes the nature of the responses measured. The designations by which the lines are known in the laboratory, which for the sake of clarity are not used in this paper, are given also in Table 1.

Table 1. *Summary of designations and treatments of the lines*

Designation of lines		Direction of selection	Character selected	Diet administered	
In this paper	In the laboratory			1st litter	2nd litter
H+	CFL	Upward	Growth on high plane	High	Low
H−	CFS	Downward			
L+	CRL	Upward	Growth on low plane	Low	High
L−	CRS	Downward			

Method of selection

The method of selection was strictly within litters. That is to say, two mice were selected out of the first litter of each mating, one male and one female as far as possible. This method has two advantages; it reduces the rate of inbreeding, and it reduces the influence of maternal effects on the response to selection. Twelve single-pair matings were made in each generation of every line, the matings in all lines being made contemporaneously throughout. The theoretical rate of inbreeding under this system is 1·04% per generation. Litters were standardized at birth, by reduction or augmentation, as nearly as possible to eight young, four of each sex. The intensity of selection was thus about one out of four, which gives an expected selection differential of about one standard deviation. Litters were weaned at 3 weeks, males and females being stored separately. Litters to be reared on the low plane of nutrition were fed on the low diet from 3 weeks to 6 weeks and were then fed on the normal, high diet.

This programme of selection was carried on for thirteen generations, after which the procedure was changed as follows. The second litters of generation 14 were not measured in the usual way but were used instead for measurements of food-consumption. From generation 15 onwards, rearing on low plane was discontinued, and all lines were selected for growth on high plane. At the same time the number of matings was reduced to six in each line. The line selected for small size on low plane (L−) was discontinued after generation 16. The responses in the remaining three lines are presented here up to generation 22, even though after generation 13 they are not strictly relevant to the main problem.

It would have been desirable to keep unselected lines as controls and to measure them at every generation, but this would have necessitated a reduction in the number of mice reared and measured in the selected lines, because space was limited; and it was decided—perhaps wrongly—not to keep control lines. An unselected line was, however, measured contemporaneously with generation 7. This line, known as JC, was derived from the same base population and was in use as a control for another experiment. First-litter mice were used as controls for the direct responses, and second-litter mice for the correlated responses. Thus the controls are represented on the graphs by two points at generation 7.

Diets

The high plane of nutrition was the normal cubed diet used in the laboratory. Its analysis (for which I am indebted to Dr Ruth E. Fowler) was as follows: carbohydrate, 56·8%; protein, 18·5%; fat, 4·5%; water, 12·9%; ash, 7·3%. The low plane of nutrition was provided by a specially prepared cubed diet made from the ingredients of the normal cubes 'diluted' with 50% of indigestible fibre in the form of ground oat husks. This low diet was fed *ad lib.* over the 3-week period from 3 to 6 weeks, and it reduced the growth during this period by about 20%. A batch of 14 cwt. of this special diet was manufactured at the beginning of the experiment and was used throughout generations 0–9. It is almost inevitable that some changes of its nutritional value took place during this period of storage, and the controls measured at generation 7 indicated a reduction of 10% in the growth of mice fed on the low diet when compared with the beginning of the experiment. A second batch of special diet was manufactured later and put into use at generation 10. No general effect of this change was apparent. Measurement of the food-consumption of mice on each diet will be described later.

RESULTS

If this experiment can justifiably be regarded as a 'model', showing what might be expected to happen in the practice of livestock breeding, its results are very clear in the breeding policy to which they point. But, as a test of the adequacy of current theory for the prediction of the responses, its results are less clear. For this reason the theoretical analyses of the responses will be deferred till the end of this section and the results will be presented first in the form of a straightforward description.

Description of responses

The responses to selection are shown graphically in Fig. 2*a*. (Fig. 2*b* is for later use, in the analysis of the responses.) The points in these graphs show the mean growth in each successive generation, each point being the unweighted mean of males and females. Continuous lines show the responses to direct selection (i.e. selection for the character itself) and broken lines show the responses to indirect selection (i.e. the correlated responses to selection for the other character). There are four comparisons to be made between direct and indirect selection in respect of the progress made, referring respectively to growth on high plane and to growth on low plane in response to upward and to downward selection. The results depicted in Fig. 2*a* are as follows:

1. *Increase of growth on high plane.* Direct selection was more effective than indirect selection at the beginning, but not very much more effective. Toward the end, however, the correlated response caught up with the direct response. In the long run, therefore, indirect selection was just as effective as direct selection.

2. *Increase of growth on low plane.* Not much progress was made by either

method. Direct selection, however, did yield some progress, whereas indirect selection yielded none at all.

3. *Decrease of growth on high plane.* Direct selection was much more effective than indirect selection, which yielded hardly any progress.

4. *Decrease of growth on low plane.* Direct and indirect selection were equally effective, both yielding rapid progress.

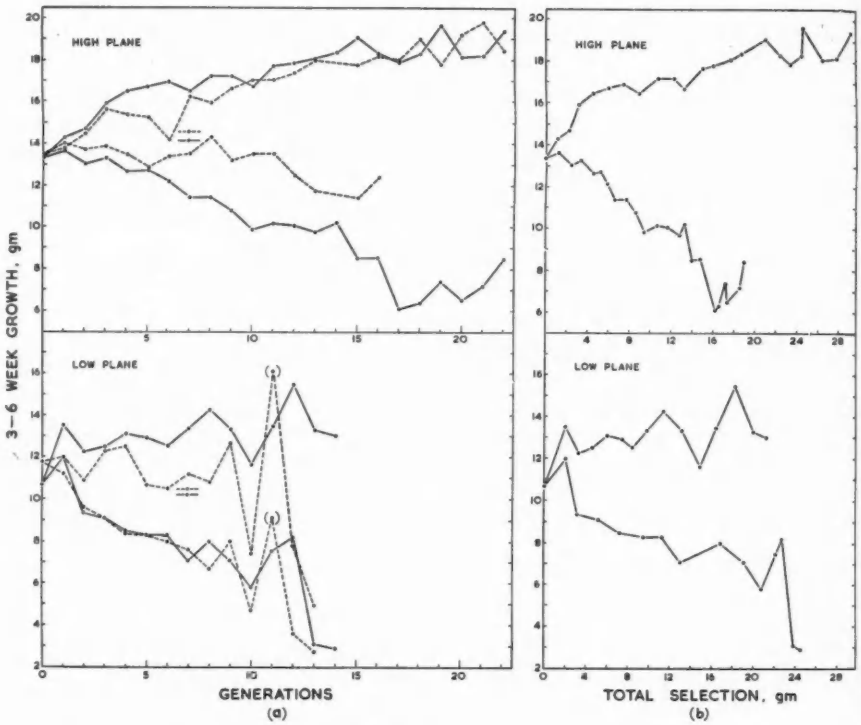


Fig. 2. Responses to selection in the four lines. Solid lines show the direct responses, broken lines the correlated responses. The horizontal lines at generation 7 are unselected controls, solid lines referring to first litters for comparison with direct responses, broken lines to second litters for comparison with correlated responses. The bracketed points are open to suspicion of error.

The responses to selection for increased growth (1 and 2 above) agree with the results of an earlier experiment (Falconer and Latyszewski, 1952) conducted on similar lines but on a smaller scale: growth on high plane was increased almost as much by selection on low plane as by selection on high plane, but growth on low plane was not increased at all by selection on high plane. In both experiments selection on high plane improved only growth on the high plane, but selection on low plane improved growth on both the low and the high planes. In practice there

must always be a certain range of environmental conditions to which any breed is subjected, and it should perform well under all. The breeding policy to which the results of these experiments point is, therefore, to subject the animals during selection to environmental conditions corresponding to the worst, rather than the best, under which the improved breed will be required to perform. This, if the results of the mouse experiments should prove to be general, will produce animals with the best all-round performance.

The first comparison made above, between the two large lines when grown on high plane, showed that direct and indirect selection were in the long run equally effective in increasing growth on high plane. This, however, is not the whole story. The mice of the low-plane line were heavier at 3 weeks, 6 weeks and at 12 weeks of age; and they were less fat than the mice of the high-plane line. Thus, indirect selection produced animals that were more desirable from the 'economic' point of view, if the mice are regarded as 'models' of farm animals. The data concerning these subsidiary aspects of the selection will be presented later.

Weaning weight

One of the advantages of the method of selection within families applied in this experiment is that no direct selective pressure is put on maternal performance. The maternal performance may nevertheless change as a correlated response following the changes of body size, and an increased or decreased maternal performance may influence the growth of the offspring as a maternal effect. Maternal effects as a possible complication of the responses of growth may be explored by an examination of the weaning weights (i.e. at 3 weeks) and of the connexion between weaning weight and subsequent growth. The weaning weights of the four lines are shown in Fig. 3. The large and small lines differentiated, as

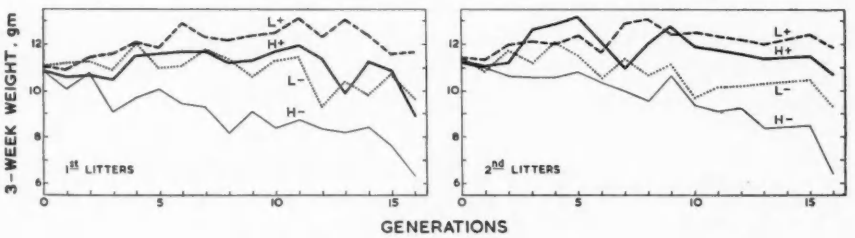


Fig. 3. Weaning weights of the four lines.

is to be expected, the large lines increasing and the small lines decreasing in weaning weight. But a more important, and unexpected, difference is found between the high-plane and low-plane lines. Both low-plane lines had higher weaning weights than the corresponding high-plane lines. The difference may have been partly a direct effect of the diet on the maternal performance of the females, because the difference is more apparent in first than in second litters, and

because some difference was already apparent in the first generations. It cannot, however, have been entirely due to a direct effect of the diet, because the difference persisted after generation 14 when all mice were reared on high plane. (Additional estimates of the weaning weights of the two large lines were obtained in generation 19 and will be presented later.)

The connexion between weaning weight and subsequent growth may be assessed from the regression of growth on 3-week weight. Regressions were calculated both within litters and between litters, the latter from the components of covariance and variance between litters. The computed regression coefficients—pooled over all generations since they showed no consistent trends— are given in Table 2.

Table 2. *Regressions of growth (3–6 weeks) on weight at 3 weeks, computed within generations and pooled over generations 0–12. The between-litter regressions are computed from the components of covariance between litters, obtained from analyses of covariance. The standard errors of the within-litter regressions are given in italics*

	Growth on high plane				Growth on low plane			
	Large		Small		Large		Small	
	♀	♂	♀	♂	♀	♂	♀	♂
Between litters								
1st litters	−0·15	0·13	0·00	0·32	0·00	0·34	0·19	0·40
2nd litters	−0·17	−0·24	−0·33	0·13	0·08	0·29	0·38	0·43
Within litters								
1st litters	0·06	0·39	0·10	0·52	0·58	0·57	0·95	0·96
	0·07	*0·08*	*0·08*	*0·07*	*0·12*	*0·12*	*0·18*	*0·19*
2nd litters	−0·05	0·31	0·03	0·54	0·93	1·23	0·94	1·01
	0·07	*0·07*	*0·07*	*0·10*	*0·15*	*0·16*	*0·14*	*0·18*

Growth on high plane was very little influenced by weaning weight, but growth on low plane was fairly strongly influenced, particularly in the within-litter component. The responses were then replotted with growth adjusted to a standard weaning weight by means of the between-litter regression coefficients; but the graphs were barely distinguishable from the uncorrected responses. It may be concluded, therefore, that the responses of growth were not materially influenced by the changes of weaning weight.

Phenotypic variation

Fig. 4 shows the phenotypic variation within litters in all four lines on both high and low planes of nutrition. The variation of growth on high plane is expressed as the coefficient of variation, since this measure proved to be independent of the mean growth. The coefficient of variation of growth on high plane was substantially the same in all the lines and remained constant at about 10% over the whole experiment. Growth on low plane, however, is less straightforward because neither

the coefficient of variation nor the standard deviation was independent of the mean growth: the large and small lines differed inconsistently whichever way the variation is expressed. The standard deviation is shown in Fig. 4, and it may be seen to have changed markedly during the course of the experiment, though not in the same way in all lines. In one line it remained fairly constant over the whole

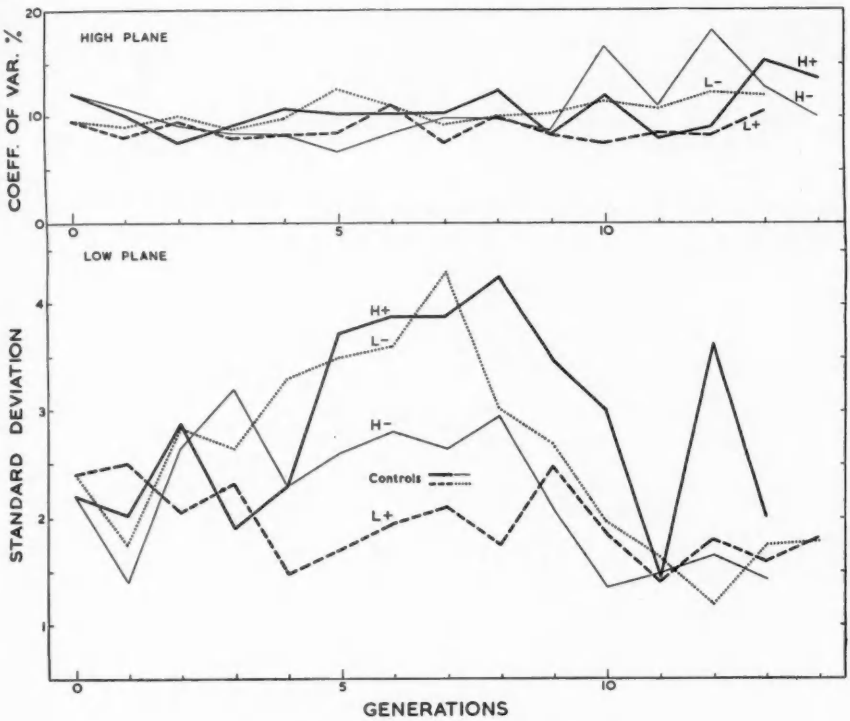


Fig. 4. Phenotypic variation within litters.

experiment. This is the line selected upwards for growth on low plane (L+). In all the other lines it increased to a maximum at about generation 7 and then decreased toward the end. The increase is most marked in the high-plane large (H+) and the low-plane small (L−) lines. It is perhaps noteworthy that these are the two lines that showed unexpectedly small correlated responses.

Susceptibility to the effect of the diet

An interesting comparison may be made between the four lines, which shows how each was affected by the low plane of nutrition. This comparison is made in Fig. 5, which shows the difference between growth on low plane and growth on high plane for each line in successive generations, the difference being expressed

as a percentage of the growth on high plane. The vertical axis thus shows the proportionate reduction of growth caused by the low-plane diet; or the 'susceptibility' of the line to the 'harmful' effects of the low-plane diet. From this viewpoint growth is regarded as a single character subject to the influence of a specific environmental difference, and the graphs show how the lines react to this environmental difference. In a limited sense they indicate environmental variability of growth. The picture presented by the graphs is clear and simple. The reaction of the two high-plane lines to the dietary difference increased over the course of the experiment, and this may reasonably be attributed to a progressive

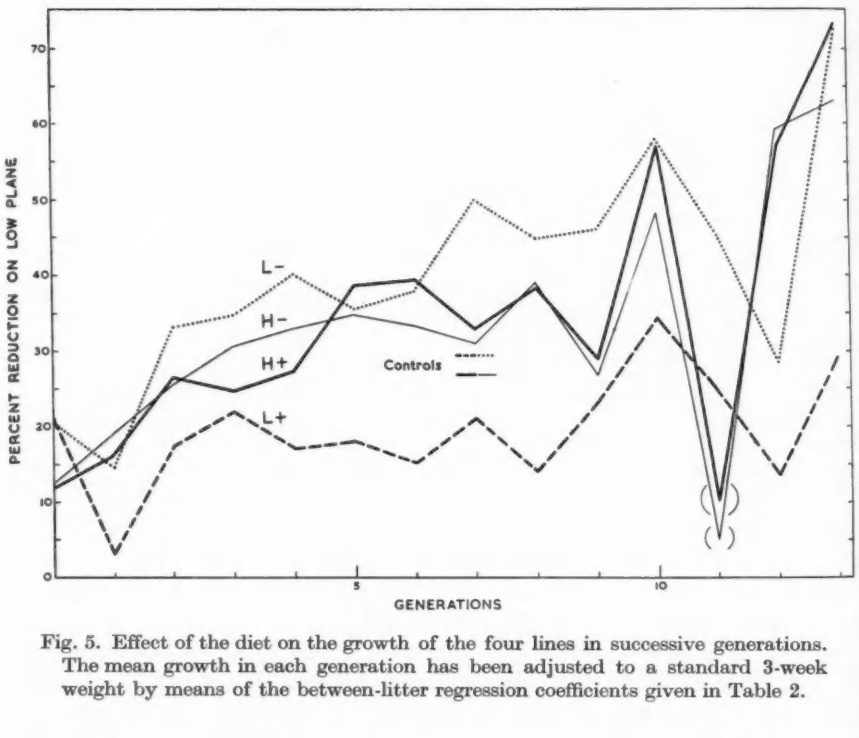


Fig. 5. Effect of the diet on the growth of the four lines in successive generations. The mean growth in each generation has been adjusted to a standard 3-week weight by means of the between-litter regression coefficients given in Table 2.

deterioration of the nutritive value of the low-plane diet. The two high-plane lines, large and small, remained virtually equal in susceptibility throughout the experiment, and we may conclude that their susceptibility was not influenced by the selection. The two low-plane lines, in contrast, changed, and changed differently. The low-plane large line (L+) decreased in susceptibility in comparison with the high-plane lines and with the controls in generation 7, whereas the low-plane small line (L−) increased in susceptibility. Selection on low plane appears therefore to have influenced environmental variability of growth, selection for increased growth having reduced it and selection for decreased growth having increased it. The

changes depicted in Fig. 5 parallel rather closely the changes of phenotypic variation of growth on low plane depicted in Fig. 4, and would do so even more closely if the variation of low-plane growth were expressed as a coefficient of variation. One can hardly avoid the conclusion that both graphs depict the same changes, namely, changes of sensitivity to differences of the dietary environment.

Food-consumption

Having presented the results of the selection we may now inquire into the nature of the two characters, 'growth on high plane' and 'growth on low plane', to which selection was applied. In particular it is desirable to know the nature of the restriction to growth imposed by the low plane of nutrition. For this purpose a small-scale study was made of the food-consumption and efficiency of gain of the four lines on both diets. The mice used for this purpose were males born in the second litters of generation 14. The mice were housed singly in cages with wood-wool for bedding but no sawdust. Every two or three days from 3 weeks to 6 weeks of age

Table 3. *Mean food consumption and efficiency of gain from 3 to 6 weeks, in males of generation 14. (The efficiencies given are means of individual ratios of gain to food consumed)*

	H+	L+	H−	L−
High-plane diet				
Number of mice	5	5	5	5
Gain, gm.	20·1	19·0	10·0	12·7
Food consumption (g).	111·0	116·1	78·7	91·6
Efficiency (%)	18·7	16·4	12·7	14·3
Food wasted (g.)	16·1	9·7	15·0	16·4
„ „ (%)	12·7	7·7	16·0	15·2
Low-plane diet				
Number of mice	4	4	0	3
Gain (g.)	1·7	11·1	—	1·1
Food consumption (g.)	67·3	109·7	—	61·7
Efficiency (%)	2·6	9·3	—	1·2
Food wasted (g.)	124·3	92·8	—	93·6
„ „ (%)	64·9	45·8	—	60·3

weights were taken of (*a*) the mice, (*b*) the unconsumed food in the food basket, and (*c*) the food wasted, i.e. unconsumed food on the cage floor after faeces and wood-wool had been removed by sieving. From these weights the gain in weight of the mice and the food consumed were obtained; and the efficiency of gain (gain in weight/food consumed) was calculated. Five mice were allocated to each group, but there were several deaths among the mice on low-plane diet, particularly among the small lines, and no mouse of the low-plane small line completed the test These deaths, which occurred in the first week, can be attributed to the mice being housed singly. For the same reason the gains were less than under the normal procedure of housing five mice per cage. The results of the feeding tests are given

in Table 3. Despite the small numbers of mice tested the conclusions about the diets are clear. It will be remembered that the low-plane diet consisted of the ingredients of the high-plane diet diluted with 50% of indigestible fibre, and that it was fed *ad lib*. The mice fed on the low-plane diet consumed less food than those fed on the high-plane, but they wasted very much more. It appears, therefore, that the mice ate selectively, rejecting the indigestible fibre. The nature of the restriction to growth imposed by the low plane of nutrition was thus in the amount, and not in the quality of the food ingested. The efficiency of gain was much less on the low than on the high plane, presumably because more energy had to be expended in getting the food. The four selected lines differ in a regular manner in their efficiency of gain. The greater the gain in weight the greater their efficiency

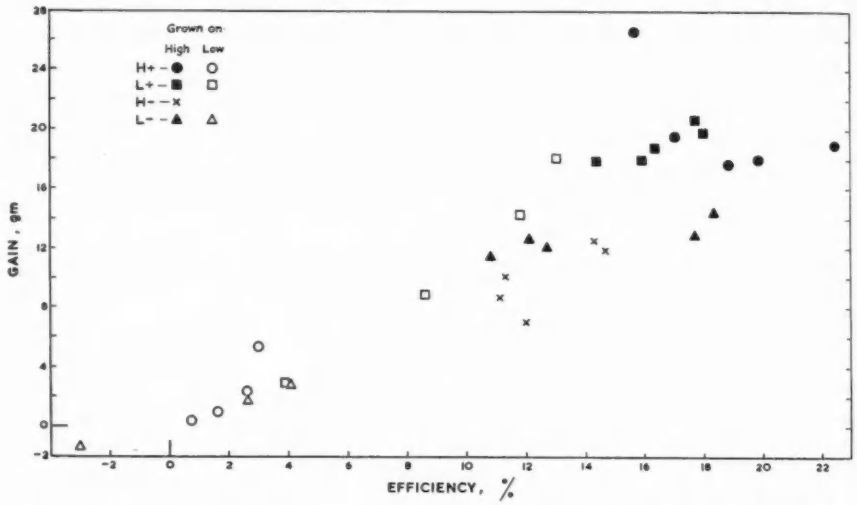


Fig. 6. Relationship between gain from 3 to 6 weeks and efficiency, i.e. gain/food-intake. Solid points refer to mice reared on high plane, open points to mice reared on low plane. Each point represents one individual.

of gain. This relationship is found also between the individuals within the groups on both diets, as shown in Fig. 6. More detailed consideration of the relationship between gain and efficiency would not, however, be justified with such small numbers of animals in each group.

Further studies of the two large lines (H+ and L+)

The responses to selection already presented showed that in the long run direct and indirect selection had been equally effective in increasing growth on the high plane of nutrition. Attention has already been drawn to the fact that this equality of growth conceals important differences between the two lines. These differences —of weight, and carcass composition—will now be described.

Body weight. In generation 19, twelve matings were set up in each of the two large lines, and most of the male offspring from both first and second litters were weighed at 12 weeks, in addition to the usual weighings at 3 weeks and 6 weeks. The weights and growth of the two lines are given in Table 4. The mice of the low-plane line were the heavier by about 3 g. at 3 weeks, at 6 weeks and at 12 weeks, though their gain in weight over the whole period was almost identical with the high-plane line. The weight of a litter at 3 weeks is more strongly influenced by the mother than by the intrinsic growth of the young. Therefore the difference of weight between the lines, appearing as it does at 3 weeks and remaining constant thereafter, must probably be attributed to a maternal effect. If this interpretation is accepted, it means that the difference in maternal performance must have arisen as a correlated response, since no direct selection was applied to maternal performance. Thus selection on low plane, though no more effective than selection on high plane when judged by the improvement of growth achieved, led to a more

Table 4. *Body weights of the two large lines, reared on high plane, measured in generation 19*

	High-plane line (H+)		Low-plane line (L+)	
	1st litters	2nd litters	1st litters	2nd litters
Number of mice	37	26	30	17
Weight (g.): 3 weeks	10·7	11·5	13·7	14·1
6 weeks	30·7	32·1	34·0	34·9
12 weeks	42·7	41·6	45·9	45·2
Growth (g.): 3–6 weeks	20·0	20·6	20·3	20·8
6–12 weeks	12·0	9·5	11·9	10·3

'desirable' produce in respect of maternal performance. The cause of the inferred difference in maternal performance very probably lies in the difference of carcass composition next to be described.

Carcass composition. Selection for large size on a low plane of nutrition was found in the previous experiment (Falconer & Latyszewski, 1952) to result in mice with less fat than those produced by selection on a high plane of nutrition. This difference was found again in the present experiment. Measurements of fatness were made on male mice of the two large lines in generations 16, 18 and 19. Rearing on low-plane diet had ceased at generation 15. The mice of generation 16 were measured at 6 weeks of age, but in generations 18 and 19 the measurements were made at 12 weeks, when the differences of fatness would be expected to be accentuated. (See Fowler, 1958.) Fatness was measured by the weight of the abdominal-fat deposit, which is easily dissected out. In addition, carcass analyses were made on a few of the generation 19 mice by Dr R. E. Fowler, who kindly allows me to quote her results. The correlation between abdominal fat and total fat was high (0·98 in the high-plane line and 0·94 in the low-plane line), so the weight of abdominal fat provides a good measure of general fatness.

The results of the measurements of abdominal fat are given in Table 5. In all of the three generations sampled the mice of the low-plane line had less fat than those of the high-plane line. The samples taken in generation 16 and 18 were rather small and the differences between the lines are not significant. In generation 19, however, the samples were larger and the difference is clearly significant. The mice measured in generation 19 were derived from eleven litters of each line, and

Table 5. *Abdominal fat of males of the two large lines, reared on high plane*

		High-plane line (H+)				Low-plane line (L+)			
				Abdominal fat				Abdominal fat	
Generation	Age	Number	Body weight (g.)	Weight (g.)	%	Number	Body weight (g.)	Weight (g.)	%
16	6 weeks	8	32·5	0·46	1·36	15	33·8	0·42	1·24
18	12 weeks	10	39·2	0·70	1·78	9	42·2	0·54	1·28
19	12 weeks	37	42·7	0·86	1·96	30	45·9	0·67	1·45

there proved to be significant differences in the percentage of abdominal fat between the litters. For this reason the test of significance of the difference between the means of the two lines was based on the observed variance of litter-means. On this basis the difference between the means is 2·4 times its standard error, which with twenty degrees of freedom has a probability of 0·02. The analyses of variance yielded the estimates of the components of variance given in Table 6. From these

Table 6. *Mean squares and components of variance of percentage of abdominal fat. Males of generation 19 at 12 weeks, all reared on high plane*

	High-plane line (H+)			Low-plane line (L+)		
	d.f.	M.S.	Component	d.f.	M.S.	Component
Between litters	10	0·785	0·127	10	0·488	0·110
Within litters	26	0·364	0·364	19	0·193	0·193
Total	36	0·481	0·491	29	0·295	0·303

figures it appears that the two lines differed in variability, particularly in the within-litter component, the low-plane line being less variable in the percentage of abdominal fat. Though the difference is significant only at the 20% level, it is probably real because a significant difference of variability was established by the carcass analyses described below, and the low-plane line was less variable, though not significantly so, also in the abdominal fat samples in generations 16 and 18.

The components of variance in Table 6 can be used to set upper limits to the heritability of the percentage of abdominal fat, though the estimates will not be at all precise when based on samples as small as these. The estimates obtained

are 52% in the high-plane line and 73% in the low-plane line. It is probable that much of the between-litter variance is environmental in origin, and the true values of the heritabilities are probably much lower than these figures.

The results of the carcass analyses made by Dr Fowler are given in Table 7. For the methods used, see Fowler (1958). These analyses were made on some of the mice of generation 19 whose abdominal fat was measured in the samples described above. In addition to the measurements of total fat, already mentioned, estimates of the water in the carcass were made as a measure of the protein content.

Table 7. *Carcass analyses of males at 12 weeks, made by Dr R. E. Fowler. All mice were reared on high plane*

	High-plane line (H+)	Low-plane Line (L+)	Significance of difference (*P*)
Number of mice	8	7	
Body weight	43·09	47·79	
Carcass weight	34·16	38·47	
Abdominal fat (g.)	0·72	0·70	
Total fat (g.)	5·47	4·53	
Water (g.)	20·24	24·39	
Total fat, as % of carcass weight	15·4	11·8	0·1–0·2
Water, as % of carcass weight	59·6	63·4	0·02–0·05
Standard deviation of % fat	4·50	1·46	0·01
Standard deviation of % water	3·82	2·10	0·2

The percentage of fat in the carcass was lower in the low-plane line than in the high-plane line, and the percentage of water, indicating protein, was higher. The latter difference is significant at the 5% level. The lower variance of the low-plane line was found again in the carcass analyses, the difference of variance of fat percentage being significant at the 1% level. In these samples the difference between the lines in the amount of abdominal fat was by chance rather less than in the larger samples referred to in Table 6. Consequently the differences of carcass composition between the lines are probably underestimated by these analyses.

Body-length and tail-length

In order to obtain some information about skeletal size, measurements of body-length and tail-length were made on some male mice of the two large lines in generation 19. The mice measured were all from second litters, and were measured at 12 weeks of age. I am indebted to Mr J. H. Isaacson for making these measurements. The means of the two lines are given in Table 8. The mice of the low-plane line had longer bodies and tails. But they were also heavier; and, since linear dimensions may be expected to vary in proportion to the cube root of weight, the comparison of the lines should be made from the ratios of body- or tail-length to the cube root of the weight. These ratios, given also in Table 8, show that the mice

of the low-plane line were proportionately longer in body and tail, but only slightly so.

Table 8. *Body-length and tail-length of males at 12 weeks, all reared on high plane*

	High-plane line (H+)	Low-plane line (L+)
Number of mice	26	18
Weight (g.)	41·6	44·7
Body-length (mm.)	108·5	113·0
Tail-length (mm.)	97·5	105·7
Body-length/(Weight)$^{\frac{1}{3}}$	31·31	31·84
Tail-length/(Weight)$^{\frac{1}{3}}$	28·14	29·78

Analysis of responses

The experiment was designed, as was explained in the Introduction, so as to provide a test of the adequacy of the theory of selection from which predictions of the responses might have been made. The prediction needed in practice is of the progress to be expected by indirect selection, relative to that expected by direct selection. For example, if growth on low plane is the character to be improved, will it be better to select for growth on high plane and achieve progress through a correlated response, or to select for growth on low plane itself and rely on the direct response? The prediction derived from the current theory of selection shows that the ratio of the improvement expected from indirect selection to that expected from direct selection should be as follows (Falconer, 1952):

$$\frac{CR_L}{R_L} = \frac{i_H}{i_L}\frac{h_H}{h_L}\mathrm{r}_A. \qquad (1)$$

(The meanings of the symbols used here and throughout this section are given in Table 9.) In the same way the expected responses of growth on high plane are given by the equation

$$\frac{CR_H}{R_H} = \frac{i_L}{i_H}\frac{h_L}{h_H}r_A. \qquad (2)$$

The best test of the validity of these equations would have been to estimate the two heritabilities and the genetic correlation in the population before selection was applied and then see if the predictions were born out. This, however, would have required a separate experiment on a large scale, and instead a less direct test was applied. All the quantities in both equations can be estimated from the observed responses to selection, except the genetic correlation. The solution of each equation therefore yields an estimate of the genetic correlation, one estimate coming from a comparison of the change of low-plane growth according to the method of selection (equation (1)), and the other from a comparison of the change of high-plane growth (equation (2)). The two estimates ought to be the same if the theory is adequate.

There is, however, a difficulty in the application of this test, which should be faced at the outset. The equations are theoretically valid for the prediction of the initial response to selection applied to a single base population. But after some generations of selection the parameters involved, i.e. the heritabilities and the genetic correlation, must be expected to change; and the theory cannot predict the extent of the changes involved. Moreover, after some generations of selection

Table 9. *Meanings of symbols*

H	the character 'growth on high plane'
L	the character 'growth on low plane'
R	direct response of the character indicated by subscript
CR	correlated response of the character indicated by subscript. (CR_H = change of H resulting from selection for L; CR_L = change of L resulting from selection for H)
i	intensity of selection (= selection differential in phenotypic standard deviations) applied to the character indicated by subscript
h^2	heritability of the character indicated by subscript
r_A	genetic correlation (i.e. correlation of breeding values) between the characters H and L

the parameters in the equations no longer refer to a single population but to two, i.e. the two lines under comparison. Therefore only the first few generations of the experiment can legitimately be used to test the validity of the theory. If the theory is upheld by the initial responses, then the later generations of the experiment can be used to answer the empirical question: how long will the prediction hold good?

Table 10. *Responses up to generation 7, judged as deviations from the control*

	Upward selection		Downward selection	
	Growth on high plane	Growth on low plane	Growth on high plane	Growth on low plane
Intensity of selection	$i_H = 0{\cdot}83$	$i_L = 0{\cdot}69$	$i_H = 0{\cdot}80$	$i_L = 0{\cdot}66$
Realized heritability	$h^2_H = 0{\cdot}26$	$h^2_L = 0{\cdot}31$	$h^2_H = 0{\cdot}42$	$h^2_L = 0{\cdot}25$
Direct response	$R_H = 2{\cdot}26$	$R_L = 3{\cdot}07$	$R_H = 2{\cdot}80$	$R_L = 3{\cdot}23$
Correlated response	$CR_H = 1{\cdot}55$	$CR_L = 0{\cdot}64$	$CR_H = 1{\cdot}17$	$CR_L = 2{\cdot}89$
Genetic correlation	$r_A = 0{\cdot}75$	$r_A = 0{\cdot}19$	$r_A = 0{\cdot}66$	$r_A = 0{\cdot}57$

Table 10 gives the data need for the solution of equations (1) and (2) from the responses up to generation 7, when a group of unselected animals were measured as controls. The responses given in the table are the total responses measured as deviations from the controls, first-litter mice being used as controls for the direct responses and second-litter mice for the correlated responses. The intensities of selection were calculated from the deviations of selected individuals from their

litter-means, weighted by the number of progeny measured in the next generation and divided by the phenotypic standard deviation within litters, males and females being averaged. The heritabilities were estimated as realized heritabilities from the ratio of total response to total selection differential. The realized heritabilities are depicted graphically in Fig. 2*b*, where the generation means are plotted against the cumulated selection differential. Four estimates of the genetic correlation between high-plane growth and low-plane growth are entered at the foot of Table 10. The estimate in each column is derived from the responses entered in the same column above it. For example, the estimate of 0·75 in the first column refers to the upward responses of growth on high plane and is obtained by equation (2) as follows:

$$r_A = \frac{CR_H}{R_H}\frac{i_H}{i_L}\frac{h_H}{h_L}$$

$$= \frac{1{\cdot}55}{2{\cdot}26}\frac{0{\cdot}83}{0{\cdot}69}\sqrt{\frac{0{\cdot}26}{0{\cdot}31}}.$$

The estimates under the columns headed 'growth on low plane' refer to the responses of growth on low plane and are obtained in a similar way by equation (1). Three of the four estimates are in reasonably good agreement, only one being seriously discrepant. This is the one derived from the upward responses of growth on low plane. Over the first seven generations of selection, therefore, we may conclude that the observed responses are in reasonably good agreement with the theory of selection for correlated characters.

For the later generations of the experiment an examination of Fig. 2*a* is sufficient to show that the responses do not agree at all well with the theory, and, in the absence of unselected controls, it would be pointless to subject them to detailed analysis. Let us therefore see if anything can be discovered about the causes of the discrepancy.

Examination of the realized heritabilities depicted in Fig. 2*b* shows that there was asymmetry between the upward and downward responses, and that the realized heritabilities of high-plane growth changed. The realized heritability of growth on high plane over the first four or five generations was about 70% when selected upwards and about 20% when selected downwards; but thereafter it was about 12% upwards and 45% downwards. Growth on low plane, apart from irregularities in the first two generations, showed a fairly constant realized heritability of about 10% when selected upwards and about 20% when selected downwards. The asymmetry of the responses may have arisen from genetic causes or from changes of the environment: the former seems more probable for the responses of high-plane growth, and the latter, through deterioration of the diet, for the responses of low-plane growth. Whatever its cause, the asymmetry could not have been predicted on the basis of theory. The next step should therefore be to find out if the discrepancies of the correlated responses can be attributed to the asymmetry.

The complications introduced by the asymmetry of the responses can, in some

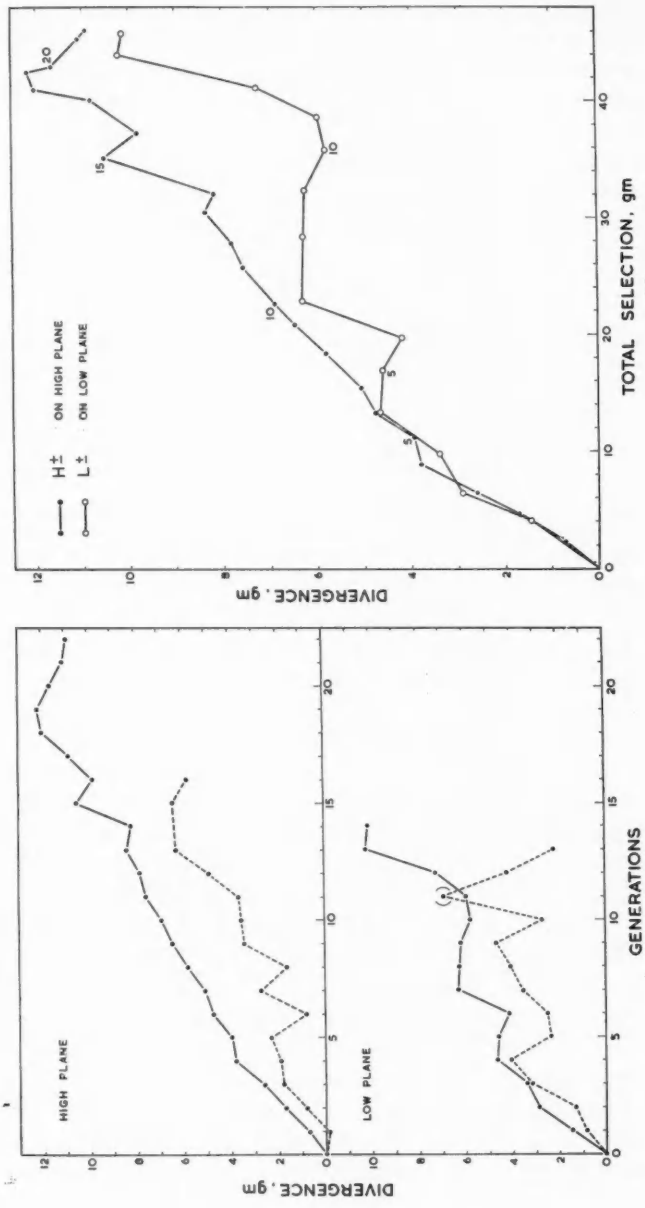


Fig. 7. Responses to selection judged from the divergence between upward- and downward-selected lines. Solid lines show the direct responses, broken lines the correlated responses. The figures alongside the graphs in (*b*) are generation numbers.

degree, be circumvented by consideration of the divergence between upward- and downward-selected lines. The responses measured as divergence are shown in Fig. 7, the generation means being plotted against generations in Fig. 7*a* and against the cumulated selection differentials in Fig. 7*b*. The correlated responses are now at least qualitatively in agreement with expectation over the whole experiment: in both cases the response to indirect selection (i.e. the correlated response) is less than the response to direct selection. This is what would be expected unless the heritabilities of the two characters, or the intensities of selection applied to them, were very different and the genetic correlation were very high.

In order to test the agreement quantitatively it is necessary to divide the experiment into parts, because neither the realized heritabilities nor the responses remained constant over the whole experiment. The responses were therefore divided at generation 4 into two parts, because this is the first point at which the heritabilities changed appreciably. Table 11 gives the results of the analyses made

Table 11. *Responses judged from the divergence between upward and downward selected lines. The columns headed 'H' refer to growth on high plane, the columns headed 'L' to growth on low plane. The genetic correlations in each column are estimated by equations (1) or (2) from the responses entered above in the same column*

	Generations 0–4		4–13		0–13	
	H	*L*	H	*L*	*H*	*L*
Intensity of selection (i)	1·66	1·40	1·65	1·52	1·66	1·48
Realized heritability (h^2)	0·41	0·36	0·22	0·13	0·30	0·20
Direct response (R)	0·90	1·20	0·53	0·46	0·69	0·70
Correlated response (CR)	0·48	0·98	0·46	−0·01	0·38	0·38
Genetic correlation (r_A)	0·67	0·65	1·25	−0·02	0·74	0·39

in the same way as was described above. Three analyses are given: up to generation 4; from generation 4 to generation 13; and over the whole experiment from generation 0 to generation 13. The realized heritabilities were computed from the regression of generation mean on cumulated selection differential and the responses from the regression of generation mean on generation number. Since the responses here are the divergence between lines selected in opposite directions, the regression lines were made to pass through the origin (i.e. no divergence at generation 0), except those referring to the period from generation 4 to generation 13.

The first four generations of selection yield two estimates of the genetic correlation that are in very good agreement with each other. Up to this point, therefore, the responses agree well with the theory of selection for correlated characters. After generation 4, however, the two characters yield widely discrepant estimates of the genetic correlation, and the responses do not agree well with the theory. Circumvention of the asymmetry between the upward and downward responses

by consideration of the divergence, though it brings the responses into qualitative agreement with the theory, does not go far towards removing the discrepancies.

The conclusion to be drawn from these analyses of the responses is that the theory of selection for correlated characters appears to be adequate for the prediction of the relative merits of direct and indirect selection over the first few generations. For the later generations, however, the theory does not adequately describe the responses. The reason for the discrepancy probably lies in the changes of the genetic parameters that took place during the course of the selection.

DISCUSSION

The conclusions drawn from the experimental results may be summarized as follows:

1. Selection for increased growth gave the same result as the previous experiment: growth on high plane was increased equally by selection on low plane as by selection on high plane, but growth on low plane was increased only by selection on low plane. The best 'all-round' performance was thus produced by selection on low plane.

2. Also in agreement with the previous experiment was the difference in carcass composition: the mice whose growth had been increased by selection on low plane were less fat than those whose growth had been increased by selection on high plane. The equality of growth was therefore reached by different physiological pathways.

3. Selection for decreased growth gave results that 'mirror-imaged' the results of selection for increased growth: growth on high plane was reduced only by selection on high plane, but growth on low plane was reduced equally by selection on high plane and selection on low plane. The best 'all-round' performance was thus produced by selection on high plane.

4. The results of selection in both directions may be generalized by saying that if good performance under a variety of conditions is desired, then selection should be made under the conditions *least* favourable to the desired expression of the character: if an increase is desired, the selection should be made under conditions that reduce the character, and vice versa.

5. These results, which refer to the final outcome of thirteen generations of selection, do not accord well with the theory of selection for correlated characters. Over the first few generations, however, the results were in reasonably good agreement with the theory. The discrepancies probably arose from changes of the genetic parameters which took place during the course of the selection.

How much reliance can be placed on these conclusions? In the first place, the absence of replicates must cast some doubt on the generality of the results, because little is known about how much the response to selection may be influenced by the special circumstances of a particular line or a particular experiment. The uniformity of response among the replicates of an experiment with *Drosophila* (Clayton, Morris & Robertson, 1957) was quite enough over the first ten generations

or so to justify the drawing of conclusions about the direct responses in an experiment without replicates, such as the present one. But the correlated responses may be less reliable, for reasons explained by Clayton, Knight, Morris & Robertson (1957), and the conclusions drawn from them—which are the main conclusions of the experiment—must be accepted only with some caution. On the other hand, selection for increased growth has produced the same results in two different experiments with mice, and the conclusions about upward selection are therefore more reliable.

In the second place, the lack of unselected controls, except at the seventh generation, weakens the theoretical analyses of the results. It does not, however, impair the reliability of the main conclusions, which are based on comparisons of two lines on the same nutritional plane.

The theoretical analyses are the least satisfactory feature of the experiment. They show that changes of the genetic parameters took place, which led to final responses that could not have been predicted from the parameters of the base population. But the precise nature of these changes and the way in which they affected the responses remain obscure. In short, the experiment shows pretty clearly *what* happened when selection was applied in two different environments, but not *why* it happened. There are, perhaps, some clues. Firstly, there is the asymmetry of the responses in opposite directions. The cause of this, which affects the direct responses, must be sought before we can hope to understand the correlated responses. Secondly, there are the changes of phenotypic variability, and of 'sensitivity' to the low-plane diet. These can be described by saying that selection on low plane affected the canalization of growth, whereas selection on high plane did not. But the changes of canalization need a genetical explanation no less than the changes of growth.

The problems raised and left unsolved by this experiment are partly genetical and partly physiological. I shall conclude by outlining the terms in which I believe the solutions should be sought. The concept of genetic correlation arises from pleiotropy as a property of genes. When two quantitative characters are under consideration, three classes of gene are postulated: A, genes that affect one character only; B, genes that affect both characters; and C, genes that affect the other character only. The segregation of genes of class A causes variation of the first character, class B of both characters, and class C of the second character. Selection applied to the first character changes the frequencies of the genes of classes A and B; the changes of the class B genes cause the correlated response of the second character. The physiological aspect of the problem consists of discovering how these three classes of gene affect the character. In the present experiment, for example, the genes of class A, say, affected fatness more than did those of the other two classes. A solution of the physiological problem would enable us to predict the qualitative differences between the products of selection in different environments. The genetic aspect of the problem consists of discovering how the genetic properties of the population change as the frequencies of the genes of the three classes change. Interaction between the genes of different classes seems

to be inescapable. For example, the effects of (i.e. the variation caused by) genes of class A, say, may be increased in the presence of plus alleles of the C class and reduced in the presence of minus alleles. The genetical problem is thus one of great complexity. Its solution seems hardly likely to be achieved until we have a wider empirical knowledge of the responses to selection in different environments, or of correlated responses in general.

SUMMARY

1. Two-way selection was applied to the growth of mice between 3 and 6 weeks of age when reared on a high plane of nutrition and, in another pair of lines, when reared on a low plane of nutrition. In each generation the growth of all four lines was measured on both high and low planes of nutrition.

2. Growth on the two planes of nutrition was treated as two different characters and the direct and correlated responses of each were estimated. The genetic correlation between the two characters was estimated from the responses of each of the four lines, and from the divergence between upward-and downward-selected lines. The different estimates should be the same if the theory of selection for correlated characters adequately accounts for the responses. Up to generation 7 the agreement was reasonably good, but in the later generations it was not. Four estimates of the genetic correlation up to generation 7 were: 0·75, 0·19, 0·66, 0·57.

3. There was asymmetry between the upward and downward responses, and the realized heritabilities changed over the course of the experiment; so also did the phenotypic variation. In all these respects the lines behaved differently.

4. The conclusions drawn from the final responses are summarized at the beginning of the Discussion.

5. The mice produced by selection for increased growth on low plane, but later reared on high plane, were compared with those produced by selection on high plane. Their growth was the same, but they were heavier, had less fat and more protein, and were better mothers.

REFERENCES

Clayton, G. A., Knight, G. R., Morris, J. A. & Robertson, A. (1957). An experimental check on quantitative genetical theory. III. Correlated responses. *J. Genet.* **55**, 171–180.

Clayton, G. A., Morris, J. A. & Robertson, A. (1957). An experimental check on quantitative genetical theory. I. Short-term responses to selection. *J. Genet.* **55**, 131–151.

Falconer, D. S. (1952). The problem of environment and selection. *Amer. Nat.* **86**, 293–298.

Falconer, D. S. & Latyszewski, M. (1952). The environment in relation to selection for size in mice. *J. Genet.* **51**, 67–80.

Falconer, D. S. & Robertson, A. (1956). Selection for environmental variability of body size in mice. *Z. indukt. Abstamm.- u. VererbLehr.*, **87**, 385–391.

Fowler, R. E. (1958). The growth and carcass composition of strains of mice selected for large and small body size. *J. agric. Sci.*, **51**, 137–148.

Genet. Res., Camb. (1960), **1**, *pp.* 114–128
With 2 text figures
Printed in Great Britain

Heterokaryon formation in *Coprinus lagopus*

By K. M. SWIEZYNSKI* and P. R. DAY
John Innes Horticultural Institution,
Bayfordbury, Hertford, Herts.

(*Received* 27 *July* 1959)

INTRODUCTION

In *Coprinus lagopus* mating type is determined by two independently inherited factors *A* and *B* (Hanna, 1925). Two haploid strains with different alleles at the *A* and *B* loci are compatible and when crossed form a dikaryon characterized by the presence of clamp connexions between adjacent cells. Each cell contains two nuclei, one from each parent mycelium. On suitable media the dikaryon produces fruit-bodies bearing haploid basidiospores.

In the related species *C. fimetarius* strains having a common *B* factor may form heterokaryons (Quintanilha, 1933). The same was also found in *C. lagopus* by Lewis & Day (1958). They further showed that common *B* heterokaryons composed of complementary auxotrophic mutants were prototrophic on minimal medium and possessed hyphal tips containing both types of nuclei. No record is known of common *A* heterokaryons or heterokaryons with both mating-type factors in common, although these have been reported in other basidiomycetes (Papazian, 1950; Raper & San Antonio, 1954; Fulton, 1950).

The present study was undertaken to analyse the formation, stability, growth and fruiting ability of various heterokaryons in *C. lagopus*.

MATERIALS AND METHODS

Four wild-type strains with different mating types were isolated from a single fruit-body growing on manure at Bayford, Herts, in 1956. The four mating types were A_5B_5 (H_1), A_6B_5 (H_2), A_5B_6 (H_6) and A_6B_6 (H_9). Three mutants, originally induced in H_9 by Anderson (1959), namely, G2212 (*chol*-1), G2242 (*ad*-8) and G1905 (*me*-5), were each crossed with H_1 and the other three recombinant mating types were obtained (i.e. A_6B_5 *chol*-1, A_5B_6 *chol*-1, A_5B_5 *chol*-1 etc.). The relative growth-rates of these mutants compared with wild type are given in Table 6. Certain other mutants and wild-type strains with different alleles at *A* and *B* have also been used occasionally.

The presence of particular nuclei in the mycelia of heterokaryons was established either by testing samples for ability to grow on a suitable medium, or by mating

* The senior author was supported by a Polish Academy of Sciences scholarship while on leave of absence from the Institute of Genetics, Skierniewice, Poland.

samples with appropriate tester stocks and scoring clamp formation. Sometimes both methods were used.

All the experiments, unless otherwise stated, were performed at 28° C. in Petri dishes 100 mm. in diameter on Fries (1953) minimal medium and complete medium described by Day (1959). The complete medium was modified by the addition of 1·25 ml. hydrolysed nucleic acid to each litre. Fruit-bodies were produced on sterilized manure.

The terminology of Papazian (1950) and Raper (1953) has been adopted. Heterokaryons with nuclei possessing the same alleles at one or both mating-type loci are called common *A*, common *B* and common *AB* heterokaryons respectively. Heterokaryons in which the alleles are different at both loci are called compatible heterokaryons or dikaryons.

The formation of compatible, common A, *common* B *and common* AB *heterokaryons*

The three mutants (*chol*-1, *ad*-8 and *me*-5) each represented by four strains of different mating types were intercrossed on minimal medium in all forty-eight possible combinations of strains with different growth requirements. The crosses were made by placing inocula of *ca.* 1 mm. side on minimal medium so that they were touching. These were incubated at 40° C. Preliminary experiments had shown that heterokaryon formation occurs more quickly at 40° C. than at 28° C. All combinations of strains (i.e. 12 common *AB*, 12 common *A*, 12 common *B*, and 12 compatible) developed and showed much better growth on minimal than the very limited growth of the parental strains. After 5 days' incubation at 40° C. most combinations had formed colonies at least 15 mm. in diameter and samples of *ca.* 1 mm. side were tested for growth requirements. Two sites in each colony were sampled, and from each site inocula were transferred to three test media: minimal plus choline, minimal plus adenine, and minimal plus methionine. If all three samples from one site grew, both parental nuclei were assumed to be present. If only one sample grew, only one parental nucleus was present and was characterized by the supplemented medium on which growth occurred.

The samples from compatible and common *B* mycelia developed on all media, showing that they contained both parental nuclei. In seven out of the twelve common *AB* crosses, samples from one or both sites gave only one type of nucleus. The same was true of five of the common *A* crosses, showing that if these common *AB* and common *A* combinations were heterokaryons they had areas in which only one type of nucleus was present. The next step was to determine whether both types of nuclei may be found in the same hypha. In combinations of compatible strains the regular formation of true clamps has always been associated with a binucleate condition in our experience. In other common *B* combinations Lewis & Day (1958) have shown that hyphal tip isolates from balanced common *B* heterokaryons give rise to prototrophic growths. The hyphal tip analysis was therefore limited to common *A* and *AB* heterokaryons.

The most vigorous common *A* and common *AB* combinations on minimal were

selected and single hyphal tips of not more than a few cells were transferred to complete medium. Five or ten tips were taken from each of four common *A* combinations and three common *AB* combinations. Forty-five out of sixty tips grew into colonies which were then tested for growth on minimal medium. The results from two common *AB* and two common *A* combinations which gave rise to colonies showing growth on minimal medium are given in Table 1. In addition,

Table 1. *The growth of hyphal tip isolates from common* AB *and common* A *heterokaryons*

Heterokaryon number	Parental strain requirements	Mating-type factors in common	Isolated	Number of hyphal tips: Growing on complete medium	Number of hyphal tips: Growing on minimal medium
9	*chol/me*	A_6B_5	10	9	9
10	*ad/me*	A_6B_6	5	5	1
18	*chol/me*	A_5	5	2	1
23	*chol/me*	A_6	10	10	10
		Total	30	26	21

all samples from common *A* heterokaryons able to develop on minimal medium were crossed with appropriate *B* tester stocks. This test confirmed the presence of two types of nuclei in each example. The same test could not be applied to the common *AB* combinations. However, one hyphal tip culture derived from heterokaryon No. 10 and four hyphal tip cultures derived from heterokaryon No. 9 were crossed with a compatible wild-type monokaryon. The resulting five dikaryons were fruited and the badisiospores tested for the segregating auxotrophic marker genes present in the original common *AB* combination. Of twenty basidiospores from the cross with No. 10, ten were prototrophic, eight were adenine-requiring, and two could not be classified owing to poor growth. None were methionine-requiring. The crosses with No. 9 showed poor basidiospore germination, but in general agreed in showing segregation of one or other but not both of the heterokaryon markers. The results suggest that the prototrophic growth of the common *AB* heterokaryon was not due to the presence of prototrophic nuclei arising by back mutation or somatic recombination and that each dikaryon contained only one type of nucelus from its common *AB* heterokaryon parent.

Evidence has been presented that two types of nuclei may be present in the same hypha in common *A* and common *AB* combinations. We propose to call all prototrophic combinations heterokaryons, but it is clear that many hyphae in these heterokaryons may have only one type of nucleus. The nuclear ratios have not been investigated. The evidence for the existence of common *B* heterokaryons has already been discussed, and in the succeeding sections of this paper the stability, growth-rates and fruiting ability of these three types of heterokaryon and the compatible heterokaryon will be discussed.

In another paper (Swiezynski & Day, 1960) it is shown that common *A* heterokaryons between mutant or wild-type strains are formed easily on minimal and complete media. The same is true of common *B* heterokaryons if suitable techniques are used. Nuclei are unable to migrate in a mycelium with resident nuclei having the same *B* allele. When two monokaryotic mycelia with common *B* alleles meet, an area of dense mycelium is frequently formed. This dense mycelium is composed partly of the common *B* heterokaryon which is unable to grow through the surrounding monokaryons. When transfers are made from the zone of contact, heterokaryotic hyphae with clamps or false clamps have always been recovered. False clamps fail to fuse with their adjacent cells whether this dense mycelium is formed or not. In some cases only a small proportion of the hyphae show clamps. In the common *B* heterokaryons studied, both true and false clamps were observed, sometimes on the same hyphae. False clamps were also seen on some dikaryons, but much less frequently than on common *B* heterokaryons. No clamps of any kind were seen on common *A* or common *AB* heterokaryons.

The stability of heterokaryons

It was shown in the previous section that in samples of *ca.* 1 mm. side of common *A* and common *AB* heterokaryons sometimes only one type of nucleus could be detected. This finding suggests that these heterokaryons are unstable and readily

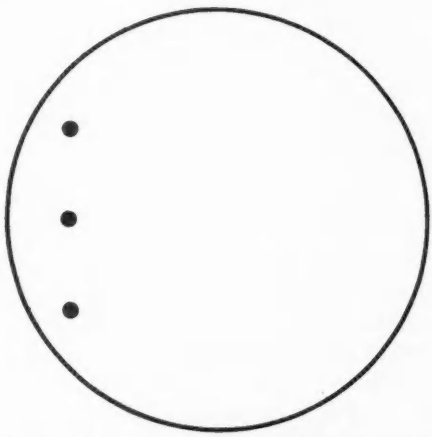


Fig. 1. Positions of the three inocula of a heterokaryon used in tests of stability and growth measurements.

break down to form their component monokaryons. The following experiments were designed to check this possibility.

The heterokaryons were first subcultured to minimal and complete medium in Petri dishes. Each dish received three inocula of *ca.* 2 mm. side, from the same source culture. They were placed as shown in Fig. 1. Compatible and common *B*

heterokaryons were mainly tested on complete medium which would support the growth of component monokaryons if they were formed by breakdown of the heterokaryons. The five common *A* heterokaryons tested were among the least stable. After the colonies had grown for 15 days samples of 1 mm. side were taken from the margins and subcultured to minimal plus choline, minimal plus adenine and minimal plus methionine. Compatible, common B and common *AB* heterokaryons were sampled at two sites where growth was weakest. Common *A* heterokaryons were sampled at four sites; two from the most vigorous and two from the weakest marginal regions. There were few instances (*ca* 4%) in which two samples from one site grew while the third did not, showing that the distribution of nuclei was usually homogeneous within each site.

A summary of the results is given in Table 2. Both types of nuclei were recovered in all samples of compatible and common *B* heterokaryons. Very few of the common *AB* heterokaryons gave samples with both nuclei present, while from 45 to

Table 2. *Stability of heterokaryons on minimal and complete media*

	Number analysed		Nuclei recovered	
Heterokaryon	Crosses	Samples	Both	One
		on minimal medium		
Compatible	2	4	4	—
Common *B*	2	4	4	—
Common *A*	5	20	17	3
Common *AB*	8	16	4	12
		on complete medium		
Compatible	12	24	24	—
Common B	12	24	24	—
Common *A*	5	20	9	11
Common *AB*	11	22	2	20

85% of the common *A* heterokaryon samples gave both nuclei. In common *A* and common *AB* heterokaryons both nuclei were recovered more frequently in samples from minimal than in samples from complete medium.

A further experiment was carried out to see if the medium may influence which nuclei are lost in the five common *A* heterokaryons. At the same time a further check was made on the stability of ten common *B* heterokaryons. Each heterokaryon was inoculated as in Fig. 1 to two plates of minimal medium each supplemented with a requirement of one of the two component nuclei. It was expected that on a medium able to support growth of only one component of a heterokaryon the other component would be the one to be lost most frequently.

After 15 days' incubation samples of *ca.* 1 mm. side were taken from the colony margins and transferred to the three kinds of supplemented minimal medium. A summary of the results is given in Table 3.

Where only one type of nucleus was recovered, with two exceptions, this nucleus was the one with requirements covered by the source medium. The

Table 3. *The stability of common* A *and common* B *heterokaryons on supplemented media*

Type of heterokaryon	Number analysed: Crosses	Number analysed: Samples per cross	Component requirements	Media supplemented with: Choline: both	Choline: *chol*	Choline: *ad*	Choline: *me*	Adenine: Nuclei recovered in samples: both	Adenine: *chol*	Adenine: *ad*	Adenine: *me*	Methionine: both	Methionine: *chol*	Methionine: *ad*	Methionine: *me*
Common *A*	2	4	*chol/ad*	5	1	2	×	5	—	3	×	—			
	2	2	*chol/me*	2	2	×	—					—	—	×	4
	1	4	*ad/me*					3	×	1	—	1	×	—	3
Common *B*	3	2	*chol/ad*	5	1	—	×	4	—	2	×				
	3	2	*chol/me*	6	—	×	—					6	—	×	—
	4	2	*ad/me*					8	×	—	—	4	×	—	4
			Total	18	4	2	—	20	—	6	—	11	—	—	11

exceptions, two adenine-requiring samples obtained from a common *A* heterokaryon between *chol*-1 and *ad*-8 growing on minimal plus choline, were both from the same heterokaryon.

When each cross in Table 3 was analysed separately it was found that in all five common *A* heterokaryons and in one common B heterokaryon either nucleus could be lost depending on the kind of medium.

Several experiments of this kind have been made on a small scale with similar results. An interesting example was provided by a common *A* heterokaryon colony growing on complete medium one area of which had only adenine-requiring nuclei and another area only choline-requiring nuclei. Occasionally one type of nucleus was recovered with a requirement not satisfied by the medium, as in the

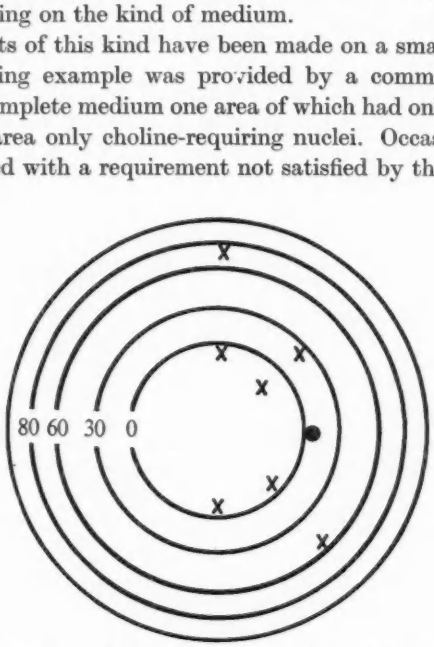


Fig. 2. Positions of the established colony margin 0–80 hours after addition of common *A* monokaryon inoculum, ●. The seven sampling points are shown X.

above example with adenine nuclei in a common *A* heterokaryon. Our findings show that the selective effect of the medium is not great and does not always operate in the direction expected.

In common *B* heterokaryons the loss of one type of nucleus was less frequent, and the resulting monokaryon was usually found as a definite sector. Such sectors would be expected as there is no migration of common *B* nuclei. In common *A* heterokaryons the loss of one type of nucleus was frequently observed, not in sectors but in areas, usually visible macroscopically as regions with weaker growth. This fact indicates that migration of common *A* nuclei may be restricted or delayed. The problem was more closely analysed in two series of experiments involving wild-type strains. In the first series an inoculum of one strain was placed at the edge of an established 4-day-old colony of a different strain, between 35 and 40 mm. in diameter, growing on complete medium. Seven points shown in Fig. 2 were selected for sampling, and from them samples of *ca.* 1 mm. side were taken after 30, 60, 80, 100 and 120 hr. and after 10 days from the time of adding the

inoculum. The samples were tested with appropriate stocks which would only be dikaryotized by the nuclei of the added strain. Migrating nuclei never disappeared from an area they had once reached. Thus the loss of nuclei of one type in a common A heterokaryon is due not to elimination of nuclei from a definite place but to the fact that they are unable to establish themselves in new growth. Such new growth may be mainly initiated by hyphae containing only one type of nucleus. In a second series of experiments, common *A* heterokaryons between wild-type stocks, in which it was known which nucleus came from the established colony and which nucleus had migrated, were inoculated as in Fig. 1. Four different combinations were available ($A_5B_5 \times A_5B_6$, $A_5B_6 \times A_5B_5$, $A_6B_6 \times A_6B_5$ and $A_6B_5 \times A_6B_6$). After 8 days' incubation hyphal tips were isolated from the margins of the colonies and the colonies they gave rise to were checked for the presence of one or both nuclei by mating with appropriate tester stocks. The results are presented in Table 4. Both nuclei were seldom recovered together, but in 30% of the cases the nucleus which remained was the migrant one.

Table 4. *Hyphal tip analysis of common* A *heterokaryons*

Allele in common	Number of tips isolated	Type of nucleus		
		Both	Resident	Migrant
A_6	26	1	16	9
A_5	26	1	18	7

We find that the common *A* heterokaryon frequently has hyphal tips and areas of mycelium in which only one nucleus, resident or migrant, is present. These monokaryotic areas are free from the other nucleus, whose migration therefore seems to be limited. We propose to call this phenomenon secondary limitation of migration. It is a property of established common *A* heterokaryons, and we would distinguish it from primary limitation, which prevents or retards the establishment of heterokaryons.

Rate of growth of various heterokaryons

All forty-eight combinations of marker strains described on p. 115, all combinations of the original wild-type strains and all single marker strains and wild types were inoculated to Petri dishes as in Fig. 1 and their growth measured every 3 days. The experiment was run in two series, one on minimal and the other on complete medium. On each plate the distance from the inoculum to the colony margin (radius) of each of the two lateral colonies was measured. This method does not take into account variation in the density of growth which could result in colonies with the same absolute growth-rate having different radii.

Most colonies of compatible and common *B* heterokaryons were able to cover the entire plate after prolonged incubation up to 18 days. Other colonies and

most common *A* and common *AB* heterokaryons ceased to develop after initial normal growth. The marginal hyphae of these colonies were often found to be abnormal when inspected under the microscope. Colonies either remained in this state or after a few days commenced new but usually weaker growth.

Average values of the largest radii from each plate, measured after 9 days for the various groups of heterokaryons and parental strains, are presented in Tables 5 and 6. Table 7 shows the variation in colony size within and between groups of

Table 5. *Average maximal radius (mm.) of various heterokaryons after 9 days of growth*

Requirements of parental strains	Number of heterokaryons tested	Compatible	Common *B*	Common *A*	Common *AB*
		Complete medium			
chol/ad	4	32	40	27	25
chol/me	4	41	45	34	38
ad/me	4	39	36	26	23
Average		37	40	29	29
Wild type	1	69	48	37	—
		Minimal medium			
chol/ad	4	25	25	12	17
chol/me	4	28	27	20	28
ad/me	4	30	26	16	14
Average		28	26	16	20
Wild type	1	42	38	30	—

Table 6. *Average maximal radii (mm.) of parental strains after 9 days*

	Choline	Adenine	Methionine	Average	Wild type
Complete medium	34	34	34	34	49
Minimal medium	9*	3	7	6	47

All four mating types of each parental genotype were measured.

* Growth of choline requiring mutants on minimal medium is very faint.

heterokaryons and between colonies on the same plate. The variation between groups is presented directly. The variation within groups is expressed as the standard deviation (between plates) of each group. Each component of the group was the average of the two radii measured on each plate. The variation within plates is presented as the average difference between the radii of the largest and smallest colony on each plate.

The data for wild-type and for mutant strains are presented separately. All possible combinations were analysed. The four wild-type common *A* heterokaryons each have a different mother strain (as indicated on p. 121), and, of the six compatible heterokaryons, four have different mother strains and in two the

Table 7. *Growth variation in heterokaryons*

Type of mycelium	Wild-type strains				Auxotroph strains			
	Number measured	Average radius (mm.)	Standard deviation between plates	Average differences between colonies on same plate	Number measured	Average radius (mm.)	Standard deviation between plates	Average differences between colonies on same plate
				Complete medium				
Parental strains	4	48	4·1	0·2	12	31	8·2	4·8
Compatible	6	67	12·3	2·8	12	44	14·3	4·3
Common *B*	2	46	1·4	4·0	12	39	7·5	4·3
Common *A*	4	34	5·0	4·7	12	26	7·0	4·5
Common *AB*	–	—	—	—	12	27	10·2	2·7
				Minimal medium				
Parental strains	4	44	4·3	0·7	12	5*	3·3	1·9
Compatible	6	40	13·6	10·5	12	24	9·1	7·7
Common *B*	2	38	8·1	1·5	12	24	7·5	3·2
Common *A*	4	27	3·8	5·5	12	14	6·1	3·4
Common *AB*	–	—	—	—	12	16	9·7	7·0

* Faint growth of auxotrophic mutants on minimal medium.

mother strains were not determined. The direction of mating was not taken into account in the mutant strain combinations. Also, in common *B* combinations between wild-type strains it could not be determined.

The following conclusions may be drawn from the data presented in Tables 5–7:

1. Common *A* heterokaryons grow less vigorously than their corresponding parental strains. With wild-type strains the difference is obvious on complete and minimal medium, but with mutant strains it is visible only on complete medium.

Common *A* heterokaryons appear to grow more slowly on minimal medium than common *AB* heterokaryons (Table 5).

2. Common *B* heterokaryons are approximately as vigorous as compatible heterokaryons and much more vigorous than common *A* heterokaryons.

3. The compatible heterokaryon usually grows fastest, but there are two exceptions:

(*a*) On minimal medium the wild-type compatible heterokaryons are not as vigorous as the haploid component strains and are much less vigorous than when grown on complete medium (Table 7).

(*b*) On complete medium compatible heterokaryons show the greatest variation in vigour (Table 7); indeed, among the twelve compatible heterokaryons there were two which showed less growth than the average growth of their parental monokaryons.

Fruit-body formation

The ability of the heterokaryons to produce fruit-bodies has been tested in two kinds of experiments:

The first experiments began as an attempt to induce mutations at the *A* or *B* mating-type loci. Mixed complementary auxotrophic strains with common *A* or common *B* factors were grown on minimal medium in Petri dishes and irradiated with ultra-violet light in various doses. The Petri dish cultures were then transferred to flasks of dung and incubated. Any mutations leading to full compatibility were expected to give rise to a prototrophic dikaryon and hence to a fruit-body when inoculated to dung.

In the other experiments heterokaryons between mutant and wild-type strains and wild-type monokaryons were inoculated to dung flasks without treatment.

The data from these experiments were supplemented with data obtained from current laboratory work with wild-type strains and mutants in compatible heterokaryons.

The results obtained are summarized in Table 8. No haploid strains produced fruit-bodies, but the number tested was limited to four wild-type strains, each in two replications. Compatible heterokaryons invariably produced fruit-bodies after 8–15 days. Common *B* fruit-bodies were obtained several times, most frequently from untreated heterokaryons, and common *A* fruit-bodies were obtained three times, two from treated and one from untreated heterokaryons.

Spores were analysed in tetrads from two common B fruit-bodies, one from a

treated heterokaryon, the other from an untreated one. Both gave normal segregation for *A* alleles. There was also evidence of the segregation of a new *B* reaction in both fruit-bodies.

Table 8. *Fruit-body formation by various mycelia*

Heterokaryon	Number of combinations	Total number of flasks	Number of flasks fruited	Days kept	Days to fruit
Compatible	9	21	21	—	8–15
Common *B* treated	10	33	1	40–90	12
Untreated	5	11	7	40–90	10–35
Common *A* treated	4	20	2	50–90	33–43
Untreated	10	13	1	50–60	17
Monokaryons	4	8	0	50	—

Spores from all three common *A* fruit-bodies obtained were analysed. None of the spores from the first fruit-body could be germinated. The second fruit-body (A_6B_5 *me*-5 × A_6B_6 *ad*-8) was contaminated and only four spores were recovered. Three were A_5B_6, and the fourth produced clamp connexions spontaneously. Twenty-nine spores were analysed from the third fruit-body ($A_5B_5 \times A_5B_6$, wild types untreated); five were A_5B_5 and six were A_5B_6. The remaining eighteen cultures all produced clamps spontaneously, but, except for two, could be assigned to B_5 or B_6 since they were only able to dikaryotize one of these testers. All eighteen cultures were able to dikaryotize the A_5 tester.

When examined again, the culture with clamps from the second fruit-body also behaved in this way and was assigned to B_5.

The new *A* and *B* reactions found suggest that fruiting may be a result of mutation in the heterokaryons.

DISCUSSION

From the foregoing data we are now able to draw a fairly clear picture of heterokaryon formation in *C. lagopus*. All types of heterokaryon—compatible, common *A*, common *B* and common *AB*—may be formed and, as was shown elsewhere (Swiezynski & Day, 1960), nuclear migration occurs in matings between strains with common *A* factors as well as in compatible matings. Two important features of these heterokaryons emerge: (1) that different *B* alleles are necessary for nuclear migration, and (2) that different *A* alleles are necessary for synchronous nuclear division and clamp formation. These features imply a functional difference between the two loci determining mating type.

C. lagopus shows many similarities with other tetrapolar basidiomycetes. Migration of nuclei in common *A* matings resulting in the formation of common *A* heterokaryons has been observed in *Schizophyllum commune* (Raper & San Antonio, 1954; Snider & Raper, 1958) and in *Cyathus stercoreus* (Fulton, 1950). In the

latter fungus, Fulton has claimed that the B locus controls nuclear migration. The common *A* heterokaryon of *S. commune* is similar to that of *C. lagopus* in showing weaker growth than a dikaryon and in giving rise mainly to monokaryotic colonies when subcultured from single hyphal tips (Papazian, 1950; Raper & San Antonio, 1954). The apparent discrepancies between *C. lagopus* and *S. commune*: that the common *A* heterokaryon of *S. commune* is sterile, does not show genetic complementation, and tends to produce morphological mutants (heterokaroytic mutagenesis), may be dependent on the genetic background and unconnected with the *A* locus itself.

Common *B* heterokaryons with pseudo-clamps have been described in *Coprinus fimetarius* by Quintanilha and in *Cyathus stercoreus* by Fulton. They are probably produced in many other forms (Nobles *et al.*, 1957). In *C. stercoreus* nuclear migration does not occur in common *B* matings. In *S. commune* the common *B* heterokaryon has only been analysed to a limited extent (Papazian, 1950). It appears to be difficult to obtain and has no clamps.

Examples of common *AB* heterokaryons are scarce. They have been tentatively described in *S. commune* (Raper & San Antonio, 1954, and in *C. stercoreus*, *loc. cit.*), in which they are described as having some uninucleate cells with false clamps.

From the foregoing discussion there are indications that the differentiation in function between the *A* and *B* loci which we have postulated in *C. lagopus* may be found in other basidiomycetes. However, this idea must be tested extensively before valid generalizations may be made.

Our analysis of the various heterokaryons reveals some further features. Thus, although nuclear ratios in common *A* heterokaryons of *Coprinus* have not been determined, the selective action of supplemented media (Table 4) shows that the relative proportions of component nuclei may change in either direction. The response of common *B* heterokaryons to such selection pressures is very limited.

A comparison of the growth rates of the heterokaryons is instructive. It shows that under some circumstances the prescribed nuclear ratio of compatible heterokaryons places them at a disadvantage compared with their component monokaryons. (Compare Tables 6 and 7, growth on minimal). We also find that the standard deviation of 'colony radius' shown in compatible heterokaryons is greater than that of other heterokaryons. One reason for these findings could be that if nutrient demands exceeded the capacity of the substrate to supply them, growth would slow or stop. The same thing would also happen if some harmful metabolic products were produced too rapidly. Dissociation into component monokaryons might result in continued if less vigorous growth. On the other hand, the poorer growth of common *A* heterokaryons may be due to unbalanced nuclear ratios.

We have demonstrated that common B heterokaryons may produce fruitbodies in *C. lagopus*. These have also been noted in *C. fimetarius* (Quintanilha, 1933). Fruiting in common *A* heterokaryons has not been reported before in this

organism and we know of no account in any other tetrapolar basidiomycete. Preliminary studies of the products of these fruit-bodies have proved the heterokaryotic nature of the mycelia on which they were borne, since we have shown that two *A* factors segregate from the common *B*, and two *B* factors segregate from the common *A* fruit-bodies. In both the expected lack of segregation of the original homozygous allele was complicated by the appearance of new properties which have so far not been explained.

SUMMARY

1. The four possible kinds of heterokaryon of *Coprinus lagopus* with no, one or both mating-type factors in common (dikaryon, common *A*, common *B* and common *AB*) were produced. Analysis of hyphal tips of common *A* and common *AB* heterokaryons has shown that both nuclei may be present in the same hypha.

2. All four heterokaryons are prototrophic when synthesized from two auxotrophic components with different requirements.

3. When synthesized in this way compatible heterokaryons were stable in all tests, but the other heterokaryons showed different degrees of stability. Common *B* heterokaryons were the most stable and rarely gave rise to monokaryotic mycelia. Dissociation of the common *A* and the common *AB* heterokaryon into either component took place much more easily.

4. Comparisons of the growth-rates of wild-type heterokaryons on complete medium show that common *A* heterokaryons are less vigorous, and dikaryons more vigorous than their monokaryon components. On minimal medium both compatible and common *A* heterokaryons are less vigorous than their wild-type monokaryon components. The possible reasons for this are discussed.

5. Fruit-bodies have been obtained from both common *A* and common *B* heterokaryons. Both types showed normal segregation at the heterozygous locus (*B* or *A*), but showed in addition the segregation of new reactions at the 'homozygous' locus.

REFERENCES

Anderson, G. E. (1959). Induced mutants in *Coprinus lagopus*. (Abstract). *Heredity*, **13**, 411–412.

Day, P. R. (1959). A cytoplasmically controlled abnormality of the tetrads of *Coprinus lagopus*. *Heredity*, **13**, 81–87.

Fries, L. (1953). Factors promoting growth of *Coprinus fimetarius* (L) under high temperature conditions. *Physiol. Plant.* **6**, 551–563.

Fulton, I. W. (1950). Unilateral nuclear migration and the interactions of haploid mycelia in the fungus *Cyathus stercoreus*. *Proc. nat. Acad. Sci., Wash.*, **36**, 306–312.

Hanna, W. F. (1925). The problem of sex in *Corpinus lagopus*. *Ann. Bot.* **39**, 431–457.

Lewis, D. & Day, P. R. (1958). *John Innes Hort. Instn Ann. Rep.* 1957, p. 18.

Nobles, M. K., Macrae, R. & Tomlin, B. P. (1957). Results of interfertility tests on some species of hymenomycetes. *Canad. J. Bot.* **35**, 377–387.

Papazian, H. P. (1950). Physiology of the incompatibility factors in *Schizophyllum commune*. *Bot. Gaz.* **112**, 143–163.

Quintanilha, A. (1933). Le problème de la sexualité chez les champignons. *Bol. Soc. Broteria*. (2ª serie), **8**, 100 pp.

RAPER, J. R. (1953). Tetrapolar sexuality. *Quart. Rev. Biol.* **28**, 233–259.
RAPER, J. R. & SAN ANTONIO, J. P. (1954). Heterokaryotic mutagenesis in hymenomycetes. 1. Heterokaryosis in *Schizophyllum commune*. *Amer. J. Bot.* **41**, 69–86.
SNIDER, P. J. & RAPER, J. R. (1958). Nuclear migration in *Schizophyllum commune*. *Amer. J. Bot.* **45**, 538–546.
SWIEZYNSKI, K. M. & DAY, P. R. (1960). Migration of nuclei in *Coprinus lagopus*. *Genet. Res.* **1**, 129–139.

Genet. Res., Camb. (1960), **1**, *pp.* 129–139
With 1 *plate and* 2 *text figures*
Printed in Great Britain

Migration of nuclei in *Coprinus lagopus*

By K. M. SWIEZYNSKI* and P. R. DAY
John Innes Horticultural Institution,
Bayfordbury, Hertford, Herts.

(*Received* 27 *July* 1959)

INTRODUCTION

The migration of nuclei in compatible matings between monokaryotic strains of a basidiomycete was established by Buller (1931) in *Coprinus lagopus*. Since then it has been observed in various other species and genera (Dickson, 1936; Fulton, 1950; Kimura, 1954; Snider & Raper, 1958). In *C. lagopus* when mycelium of a compatible strain is placed on one side of an established colony of a monokaryon the nuclei of the added mycelium spread within the mycelium of the monokaryon. Normally both strains in a compatible mating are able to accept and donate nuclei, but in some matings only one of the strains may accept nuclei, the other acting only as a donor. This behaviour has been called unilateral dikaryotization.

Migration of nuclei with the same allele at the *A* locus as the nuclei of the mycelium into which they pass has also been demonstrated in several basidiomycetes (Raper, 1953), but no migration of this kind has been found in *C. lagopus* (Papazian, 1958).

In another paper, Swiezynski & Day (1960) report the occurrence, and describe some of the properties of common *A*, common *B* and common *AB* heterokaryons in *C. lagopus*. This paper describes studies of nuclear migration during the establishment of these heterokaryons and the dikaryon.

MATERIALS AND METHODS

Strains derived from wild-type 68 (A_2B_1) (Day, 1959) and cultures and media described in our preceding paper (Swiezynski & Day, 1960) were used.

Matings and tests of migration were made in two ways. A plug 2 mm. in diameter, punched from a colony of one parent, was placed at the edge of an established colony, 20–40 mm. in diameter, of the other parent (Fig. 1). After given time intervals samples were taken from various parts of the established colony and tested for migrant nuclei. This method was used with reciprocal pairings for all measurements of the speed of nuclear migration. Alternatively, rectangular (1×15 mm.) inocula of the two mycelia to be mated were placed at an angle of about 45 degrees to each other, the nearest points being some 3 mm. apart (Fig. 2). Three such matings were made on a plate. After 3 or 4 days' incubation, two

* The senior author was supported by a Polish Academy of Sciences Scholarship while on leave of absence from the Institute of Genetics, Skierniewice, Poland.

samples from each parent, at points 10 and 20 mm. from the region of contact between the mycelia, were tested for migrant nuclei.

The samples were either small (*ca.* 1 mm. side) or large (*ca.* 3 mm. side). Several methods were used to detect migrant nuclei in them. When the mating was between compatible strains, dikaryotization was assumed to have taken place if clamp connexions were observed in the samples grown on fresh media. Samples from common *A* or common *B* matings were mated with a tester stock compatible with migrant nuclei but incompatible with resident nuclei. Abundant clamp formation was regarded as evidence of the presence of migrant nuclei. Samples

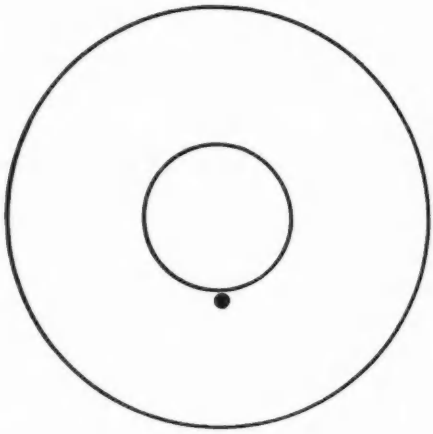


Fig. 1

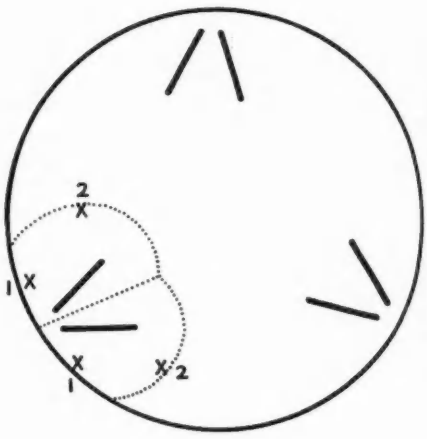
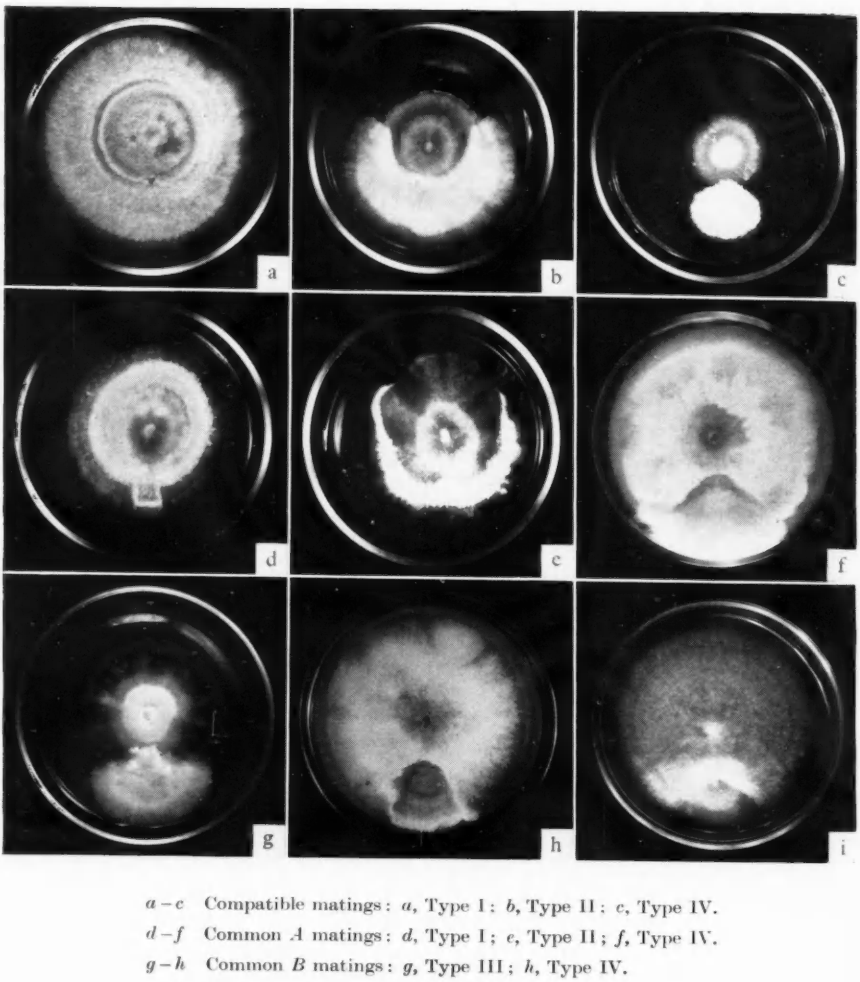


Fig. 2

Figs. 1 and 2. Methods of mating mycelia: Fig. 1: 2-mm. plug at edge of colony 20–40 mm. in diameter. Fig. 2: rectangular inocula (1 × 15 mm.) placed at an angle of about 45 degrees to each other. X, sampling points: 1, 10 mm., and 2, 20 mm. from region of contact between mycelia.

from matings between complementary auxotrophic strains were tested for prototrophy on minimal medium or on minimal medium supplemented with the requirement of the migrant nucleus only. Prototrophic growth on either medium was regarded as proof of the presence of migrant nuclei.

The occurrence of nuclear migration was not confirmed by other methods. It could be argued that what we accept as migration may be explained as enhanced growth of one mycelium through another. We think that this explanation is untenable for the following reasons: the parent mycelia in common *A* and common *AB* matings do not grow through each other. It is difficult to conceive how mycelia may grow through a dense, established, mass of hyphae in media nearly exhausted of nutrients. The most rapid nuclear migration occurs round the margins of the mated colonies (Buller, 1931), but in these areas there is no obvious invasion of hyphae.



a–*c* Compatible matings: *a*, Type I; *b*, Type II; *c*, Type IV.
d–*f* Common *A* matings: *d*, Type I; *e*, Type II; *f*, Type IV.
g–*h* Common *B* matings: *g*, Type III; *h*, Type IV.
i Common *AB* mating: Type III.

All matings were made on complete medium in Petri dishes 100 mm. in diameter and incubated at 28° C. unless otherwise stated.

The influence of mating type on the migration of nuclei

To test the migration of nuclei, compatible, common *A* and common *B* matings were made of the four wild-type strains H_1 (A_5B_5), H_2 (A_6B_5), H_5 (A_5B_6) and H_9 (A_6B_6). No common *AB* matings were made because the heterokaryons cannot be recognized unless marked strains are used. At the same time, two mutants with choline (*chol*-1), and methionine (*me*-5) requirements, each represented by the four mating-type strains A_5B_5, A_5B_6, A_6B_5 and A_6B_6, were tested in all complementary combinations (i.e. choline- and methionine-requiring strains only were crossed). The choline- and methionine-requiring strains tend to be unilateral in compatible matings and they were selected to see if they would also only donate nuclei in incompatible matings. Certain matings with other mutant strains were also tested.

The reactions observed could be divided into the following types:

Type I: Nuclei from the added strain migrate rapidly and may be found in all parts of the established colony. Before migration is complete no margin is visible between the invaded and uninvaded areas.

Type II: The added nuclei migrate more slowly than in Type I. The migration proceeds most quickly in the young mycelium and eventually extends to the whole of the established colony. There is a visible margin between the invaded and uninvaded areas. This margin does not always correspond exactly with the extent of migration, and migrating nuclei may be found beyond this limit.

Type III: A slow spread of nuclei across the region of contact which perhaps might be due to limited hyphal growth of the added strain. The extent of invasion is often greater in the older parts of the colonies than in the periphery. Differences between the invaded and uninvaded area may or may not be visible.

Type IV: No migration of nuclei occurs. Nuclei of one strain only can be detected at a distance of 5 mm. from the region of contact.

Photographs illustrating these reaction types from matings made by the method shown in Fig. 1 are shown in Plate 1.

The results obtained from matings incubated for 4 days are presented in Table 1 and may be summarized as follows:

1. In compatible matings all types of reaction (I, II, III and IV) were observed. Type III or IV reactions were found when the established colony was formed by a unilateral stock.

2. In each common *A* mating nuclear migration could be observed, but in two combinations ($A_5B_5 \times A_5B_6$ and $A_2B_5 \times A_2B_6$) it was not observed in every test. In 27 out of 46 matings migration of Type I occurred. Nuclei containing either of the three *A* alleles tested (A_2, A_5 and A_6) were able to migrate in common *A* mycelia.

3. In the crosses between choline- and methionine-requiring strains in which

Table 1. *Migration of nuclei in various mating-type combinations*

Established strain	Added strain	Compatible: Number of matings	Compatible: Type of reaction I	II	III	IV	Common *A*: Number of matings	Common *A*: Type of reaction I	II	III	IV	Common *B*: Number of matings	Common *B*: Type of reaction I	II	III	IV	Common *AB*: Number of matings	Common *AB*: Type of reaction I	II	III	IV
Wild-type-1*	Wild-type-1	12	12	—	—	—	12	8	—	4	—	8	—	—	—	8†	—	—	—	—	—
	Wild-type-2	—	—	—	—	—	6	5	—	—	1†	6	—	—	—	3†	—	—	—	—	—
Wild-type-2*	Wild-type-1	—	—	—	—	—	6	5	—	—	1†	6	—	—	—	3†	—	—	—	—	—
Wild-type-1	*me*-5 *ad*-5	1	—	1	—	—	1	—	1	—	—	1	—	—	—	1	—	—	—	—	—
,,	*ad*-5	1	1	—	—	—	1	—	—	—	1	1	—	—	—	1	—	—	—	—	—
me-5 *ad*-5	Wild-type-1	1	—	—	—	1	1	—	—	—	1	1	—	—	—	1	—	—	—	—	—
me-5	,,	—	—	—	—	—	1	1	—	—	—	1	—	—	—	1†	1	—	—	—	1†
chol-1	,,	—	—	—	—	—	2	1	1	—	—	2	—	—	—	2†	2	—	—	—	2†
ad-8	,,	—	—	—	—	—	4	2	2	—	—	4	—	—	2	2	3	—	—	1	2
chol-1	*me*-5	6	3	2	1	—	6	4	1	—	1	6	—	—	1	5	6	—	—	1	5
me-5	*chol*-1	6	1	2	3	—	6	1	2	2	1	6	—	—	2	4	6	—	—	3	3

* Wild-type-1 strains had A_5, A_6, B_5 or B_6 alleles
Wild-type-2 strains had either A_2 or B_1 alleles
† Scored after 3 days

all m
about
4.
a few
regio
altho
either

Type
Com
Com
Com
Com
main
nucl
The
sam
time
sam
seco
A
com
mai
nucl
Th
mat
also
mati
Larg
Initi
take
100
myc
com
blish
nucl
Th
sam
time
sam
seco
A
com
mai
nucl

all mating type combinations were tested, the migration of common *A* nuclei was about as frequent as the migration of compatible nuclei.

4. No extensive migration of common *B* or common *AB* nuclei was found. In a few matings the nuclei of the added strain were found a short distance from the region of contact (Type III). They were never found more than 10 mm. from it, although sometimes enhanced mycelial growth occurred more than 10 mm. on either side of the junction of mycelia (Plate I, *i*).

Comparison of the speed of migration of compatible and common A *nuclei*

The four wild-type strains A_5B_5, A_5B_6, A_6B_5 and A_6B_6 were each reciprocally mated with the compatible strains A_6B_6, A_6B_5, A_5B_6 and A_5B_5 respectively, and also with the common *A* strains A_5B_6, A_5B_5, A_6B_6 and A_6B_5 (see Fig. 1). The matings were made when the established colonies were about 40 mm. in diameter. Large samples were taken from the established colonies after 30 and 60 hours. Initially the matings were carried out on complete medium. Ten samples were taken from each colony at each time and the sampling was repeated after 80 and 100 hours. At each sampling time the radius of the colony formed by the added mycelium was also measured. This colony soon became either a dikaryon or a common *A* heterokaryon, according to the mating, forming a sector in the established colony. Nuclear migration was observed in every mating, and migrant nuclei were detected in the marginal and central parts of the invaded colonies. The rate of migration was much faster than the rate of growth of the mycelium. In samples taken from the margins of common *A* matings after 80 and 100 hours sometimes migrant nuclei were not found although they had been detected in earlier samples taken along the same radii. This behaviour suggests that there may be a secondary inhibition of common *A* nuclear migration (Swiezynski & Day, 1960).

All four combinations of compatible and common *A* matings were repeated on complete and on minimal medium. Eight small samples were taken each time, mainly from the colony margins. The growth-rates for the added mycelia and the nuclear migration rates were calculated. The results are given in Table 2.

Table 2. *Speed of migration of compatible and common* A *nuclei*

		After 30 hr.		After 60 hr.	
Type of mating	Number of colonies tested	Speed of growth (mm./hr.)	Speed of migration (mm./hr.)	Speed of growth (mm./hr.)	Speed of migration (mm./hr.)
		Complete medium			
Compatible	4	0·17–0·24	0·66–1·00	0·18–0·25	0·83–0·92
Common *A*	4	0·20–0·23	0	0·18–0·25	0·00–0·66
		Minimal medium			
Compatible	4	0·10–0·17	0·33–0·50	0·10–0·15	0·50–0·92
Common *A*	4	0·17–0·24	0	0·13–0·22	0·00–0·50

The rates given are the smallest and largest for each group of four plates

The following conclusions may be drawn:

1. The rate of migration of nuclei exceeds the rate of growth of the mycelium. It should be pointed out that the calculated rate of nuclear migration is certainly too small, for reasons discussed in detail by Snider & Raper (1958).

2. The migration of common *A* nuclei is slower than the migration of compatible nuclei.

3. There was no significant difference between the speeds of nuclear migration on minimal and on complete medium when measured after 60 hours.

Heterokaryon-monokaryon matings

This section describes the results of two series of experiments to investigate the extent of nuclear migration, in either direction, between heterokaryons and monokaryons (het-mon matings).

In the first series a variety of het-mon matings were set up using wild-type strains. The method shown in Fig. 2 was used. Samples from both parents of each mating were tested for clamp formation. Samples from some matings were also mated with tester stocks to demonstrate the presence of migrant nuclei. To simplify presentation the suffixes x and y have been introduced to denote alleles 5 or 6 at either of the mating type loci, so that when x is 5, y is 6, and when x is 6, y is 5. The suffix z denotes the alleles A_2 or B_1. Thus the two formulae $(A_5B_5 \times A_6B_5)$ and $(A_5B_6 \times A_6B_6)$ can be represented by $A_xB_x \times A_yB_x$). The results from the het-mon matings are given in Table 3.

Several of the test matings are ambiguous. Thus the first line of Table 3 shows that clamps were formed on the monokaryon in 2 out of 4 het-mon matings: $(A_xB_x \times A_yB_y) \times A_xB_y$. Since such matings are incompatible, the finding of clamps on the monokaryon component in similar test matings (next 4 lines, column 6, Table 3) cannot be taken as evidence of migration of nuclei, compatible with the tester stock, into the dikaryon. The data in Table 4, which are based on a prototrophy test for migrant nuclei, show that such migration does not occur. The cause of this anomaly, which is part of the Buller phenomenon, is referred to in detail in the discussion.

In the second series of experiments, compatible, common *A* and common *B* heterokaryons were composed of auxotrophic mutants with the same growth requirements and were therefore unable to grow on minimal medium. Monokaryons were either wild type or complementary auxotrophic mutants. Common *AB* heterokaryons were always composed of complementary auxotrophic strains and het-mon matings with them were performed on minimal medium to ensure maximum stability of the heterokaryon.

The migration of nuclei was again established by tests for the presence of clamps, and tests of the compatibility of samples with appropriate tester stocks. In addition tests were made of the ability of samples to grow on minimal medium. The results are given in Table 4.

Table 3. *Heterokaryon-monokaryon matings: wild-type strains*

Heterokaryon	Monokaryon	Number of matings	Clamps on		Number of compatible results from mating two samples of				
			Heterokaryon	Monokaryon	Heterokaryon with			Monokaryon with	
Compatible	A_xB_y	4*	4	2	—			—	
($A_xB_x \times A_yB_y$)	A_xB_z	2	2	2	A_yB_x*	1		—	
	A_yB_z	2	2	2	A_xB_y*	2		—	
	A_zB_y	2	2	2	A_xB_y*	1		—	
	A_zB_y	2	2	2	A_yB_x*	1		—	
Common *B*	A_yB_y	2	0†	2	A_xB_x	2		A_xB_y	2
($A_xB_x \times A_yB_z$)	A_xB_y	2	0†	2	A_yB_x	0		A_yB_y	2
	A_xB_x	2	0†	0	—			A_xB_y	0
	A_xB_z	2	0†	2	—			—	
	A_yB_z	2	0†	2	—			—	
Common *A*	A_yB_x	2	0	1	A_xB_y	0		A_yB_y	1
($A_xB_x \times A_xB_y$)	A_yB_y	2	0	2	A_xB_x	0		A_yB_x	2
	A_xB_z	2	0	0	—			A_yB_z	2
	A_yB_x	2	0	2	—			—	
	A_yB_y	2	0	2	—			—	

* Incompatible het-mon matings.
† Only typical common *B* heterokaryon clamps were found.

The following conclusions may be drawn from Tables 3 and 4:

1. All heterokaryons regularly donated nuclei to monokaryons which were compatible, or had only one *A* factor in common, with one or both of the heterokaryon nuclei. Appropriate matings showed that compatible, common *A* and common *B* heterokaryons were able to donate either nucleus.

Table 4. *Heterokaryon-monokaryon matings: auxotrophic strains*

Composition		Number of matings	Number of positive results: Heterokaryon		Number of positive results: Monokaryon		
Heterokaryon	Monokaryon		On minimal medium	Clamps	Mated with		Clamps
Compatible							
$A_xB_x \times A_yB_y$	A_xB_x	4	0	+	A_yB_y	4	4
,,	A_xB_x	4	0	+	A_yB_y	3	4
,,	A_yB_x	4	0	+	A_xB_x	4	4
,,	A_xB_y	4	0	+	A_xB_x	4	4
,,	A_xB_x	4	0	+	—		4
Common *B*							
$A_xB_x \times A_yB_x$	A_yB_y	3	0	—	A_xB_y	2	3
,,	A_xB_y	3	0	—	A_yB_y	2	3
,,	A_xB_x	3	0	—	A_xB_y	—	—
,,	A_yB_x	3	0	—	—		3
,,	A_yB_x	3	0	—	—		3
,,	A_xB_x	3	0	—	—		3
Common *A*							
$A_xB_x \times A_xB_y$	A_yB_x	4	—	—	A_yB_y	—	4
,,	A_yB_y	4	—	—	A_xB_x	1	4
,,	A_xB_x	4	2	—	A_yB_x	4	—
,,	A_xB_x	4	4	3	—		4
,,	A_xB_y	4	2	2	—		4
,,	A_xB_x	4	3	3	—		4
Common *AB*							
$A_xB_x \times A_xB_x$*	A_xB_y	9	Mated with A_yB_y 5	—	—		—
,,	A_yB_y	7	—	7	—		7

* These heterokaryons were prototrophic

2. Compatible and common *B* heterokaryons never accepted nuclei from the monokaryon whether compatible or not. Common *A* heterokaryons frequently accept compatible and common *A* nuclei although in some matings no migration occurred. Common *AB* heterokaryons accept compatible and common *A* nuclei.

The data in Table 4 show that in matings of the type $(A_xB_x \times A_xB_y) \times A_yB_x$ there is no migration in the heterokaryon but that migration occurs when the

monokaryon is A_zB_x. The A_zB_x monokaryon was prototrophic, and since the A_yB_x monokaryon was auxotrophic its nuclei may have had a lower potentiality for migration. To determine whether such a heterokaryon can accept an A_yB_x nucleus, four different matings were made between auxotrophic common *A* heterokaryons and prototrophic wild-type monokaryons using the two methods described above (Figs. 1 and 2). Samples were tested for clamp formation and prototrophy. Each monokaryon had nuclei compatible with one or other of the heterokaryon nuclei. The results, given in Table 5, show that migration of A_5 or A_6 nuclei in common *A* heterokaryons occurred in 7 out of 16 matings.

Table 5. *Nuclear migration into a common* A *heterokaryon from a monokaryon*

	Method used	
Mating types and requirements	1 colony per plate (Fig. 1)	3 colonies per plate (Fig. 2)
$(A_5B_5 \times A_5B_6)$ *chol* $\times A_6B_6$	+	+
,, $\times A_6B_5$	—	+
$(A_5B_5 \times A_5B_6)$ *ad* $\times A_6B_6$	—	—
,, $\times A_6B_5$	—	—
$(A_6B_5 \times A_6B_6)$ *chol* $\times A_5B_5$	+	+
,, $\times A_5B_6$	+	+
$(A_6B_5 \times A_6B_6)$ *ad* $\times A_5B_5$	—	—
,, $\times A_5B_6$	—	—

DISCUSSION

Migration of nuclei in compatible matings between monokaryotic strains of tetrapolar basidiomycetes has been found in many species since Buller's classical account of 'diploidization' in *Coprinus lagopus* published in 1931. Nuclear migration in common *A* matings has been observed in some species, notably *Schizophyllum commune* (Raper, 1953; Raper & San Antonio, 1954; Snider & Raper, 1958) and *Cyathus stercoreus* (Fulton, 1950). Our finding that common *A* migration also occurs in *C. lagopus* confirms the existence in this species of what may prove to be general behaviour. So far as we are aware, extensive nuclear migration in common *B* matings has never been recorded. It is not seen in *C. lagopus*.

We find that the dikaryon of *C. lagopus* is unable to accept compatible nuclei. Both dikaryons and common *B* heterokaryons produce clamp connexions which arise during synchronized mitotic divisions of the nuclei in the growing hyphal tips. It seems possible that the mechanism controlling this synchronized division which is called into play when nuclei having different *A* alleles are present in the same cell, may also restrict or even prevent nuclear migration.

Snider & Raper (1958) found that common *A* nuclei in *S. commune* migrate at the same rate as compatible nuclei, but are not as densely distributed. The data presented here and our results published elsewhere (Swiezynski & Day, 1960) show

that this is not the case in *C. lagopus*. In *Coprinus* the migration of common *A* nuclei is slower than the migration of compatible nuclei and there appears to be some secondary limitation of migration, perhaps connected with changes due to ageing in the recipient mycelium, which retards the movement of either nucleus into the new growth of the heterokaryon.

The first studies of dikaryon × monokaryon (di-mon) matings were made by Buller (1930, 1931). The migration of nuclei from a dikaryon to a monokaryon was termed the Buller phenomenon (Quintanilha, 1937). A great deal of interest has centred on the finding that incompatible di-mon matings of the type $(A_1B_1 \times A_2B_2) \times A_1B_2$ lead to dikaryotization of the monokaryon. Several authors (Papazian, 1950; Kimura, 1958; Crowe, 1958) have shown that the resulting dikaryon may contain both nuclei of the original dikaryon $(A_1B_1 \times A_2B_2)$ or a recombinant nucleus from this dikaryon (A_2B_1) which is compatible with the nucleus of the monokaryon. Interesting examples are cited by Kimura (1958), who found that in compatible and hemi-compatible di-mon matings with *C. macrorhizus* both nuclei of the dikaryon sometimes passed to the monokaryon, supplanting its own nucleus. An explanation of the migration of both nuclei of a dikaryon, to displace the nuclei of an established monokaryon, may be found in our studies. We have shown (Tables 1–5) that common *A* migration regularly occurs between monokaryons and from heterokaryons to monokaryons. It seems reasonable to suppose therefore that in a non-compatible mating—for example, $(A_1B_1 \times A_2B_2) \times A_1B_2$—the A_1B_1 nucleus would migrate into the A_1B_2 mycelium inoculated alongside. The common *A* heterokaryon $(A_1B_1 + A_1B_2)$ thus formed would then be able to accept the other nucleus of the dikaryon (A_2B_2), becoming dikaryotized as we have already shown may happen (Table 5). In this way the migration of both nuclei may be understood. To explain the migration of both nuclei in hemi-compatible di-mon matings—for example, $(A_1B_1 \times A_2B_2) \times A_2B_3$—we only need assume that the A_2B_2 nucleus of the dikaryon was the first to migrate and was then followed by the A_1B_1 nucleus, and the first of the two possible dikaryons to become established was $A_1B_1 \times A_2B_2$. It is to be expected that this would be a rare event since compatible nuclei tend to migrate more rapidly than common *A* nuclei. To explain the migration of both dikaryon nuclei in compatible di-mon matings (e.g. $(A_1B_1 \times A_2B_2) \times A_3B_3$), Kimura (1958) has suggested that there are different degrees of affinity between unlike alleles probably controlled by modifier genes. In our view this explanation is unnecessary.

SUMMARY

1. Four main types of interaction between paired mycelia of *Coprinus lagopus* have been defined in terms of the extent of nuclear migration.

2. Nuclear migration was demonstrated in matings between monokaryotic mycelia with common *A* alleles. No extensive migration of nuclei was found in common *B* or common *AB* matings.

3. The speed of nuclear migration in common *A* matings was slower than in

compatible matings. Migration occurred to approximately the same extent in both kinds of matings.

4. In heterokaryon-monokaryon matings compatible and common *B* heterokaryons acted only as donors. Common *AB* heterokaryons acted as donors and as acceptors of compatible or common *A* nuclei. Common *A* heterokaryons always acted as donors and frequently acted as acceptors of compatible or common *A* nuclei.

5. A simple explanation is suggested for the frequently observed fact that in an incompatible di-mon mating both nuclei of the dikaryon may migrate and eventually eliminate the nuclei of the established monokaryon.

REFERENCES

Buller, A. H. R. (1931). *Researches on Fungi*, **4.** Longmans, Green & Co., London.

Crowe, L. K. (1958). (Abstract.) The exchange of genes between nuclei of a dicaryon. *Proc. X Int. Congr. Genet., Montreal*, **2**, 61–62.

Day, P. R. (1959). A cytoplasmically controlled abnormality of the tetrads of *Coprinus lagopus*. *Heredity*, **13**, 81–87.

Dickson, H. (1936). Observations on inheritance in *Coprinus macrorhizus*. *Ann. Bot.*, **50**, 719–733.

Fulton, I. W. (1950). Unilateral nuclear migration and the interactions of haploid mycelia in the fungus *Cyathus stercoreus*. *Proc. nat. Acad. Sci., Wash.*, **36**, 306–312.

Kimura, K. (1954). Diploidisation in the Hymenomycetes. I. Preliminary experiments. *Biol. J. Okayama Univ.*, **1**, 226–233.

Kimura, K. (1958). Diploidisation in the Hymenomycetes. II. Nuclear behaviour in the Buller phenomenon. *Biol. J. Okayama Univ.*, **4**, 1–59.

Papazian, H. P. (1950). Physiology of the incompatibility factors in *Schizophyllum commune*. *Bot. Gaz.*, **112**, 143–163.

Papazian, H. P. (1958). The genetics of basidiomycetes. *Advanc. Genet.*, **9**, 41–69.

Quintanilha, A. (1937). Contribution à l'étude génétique du phénomène de Buller. *C. R. Acad. Sci., Paris*, **205**, 745–747.

Raper, J. R. (1953). Tetrapolar sexuality. *Quart. Rev. Biol.*, **28**, 233–259.

Raper, J. R. & San Antonio, J. P. (1954). Heterokaryotic mutagenesis in *Schizophyllum commune*. *Amer. J. Bot.*, **41**, 69–86.

Snider, P. J. & Raper, J. R. (1958). Nuclear migration in the basidiomycete *Schizophyllum commune*. *Amer. J. Bot.*, **45**, 538–546.

Swiezynski, K. M. & Day, P. R. (1960). Heterokaryon formation in *Coprinus lagopus*. *Genet. Res.*, **1**, 114–128.

Genet. Res., Camb. (1960), **1**, *pp.* 140–150
Printed in Great Britain

Experiments on canalizing selection*

By C. H. WADDINGTON
Institute of Animal Genetics, Edinburgh, 9

(*Received* 13 *August* 1959)

INTRODUCTION

It is a platitude to point out that natural selection is not only responsible for the elaboration of evolutionary novelties, but that it has an even more universal function in the preservation of normality by the elimination of aberrant individuals. This second type of action has been referred to as stabilizing selection (Schmalhausen, 1949). This may operate in two rather different ways (Waddington, 1940, 1957). In the first place, natural selection will tend to eliminate those alleles which in the normal environment cause the development of abnormal phenotypes; this may be called normalizing selection. A rather different type of natural selection will tend to remove those alleles which render the developing animal sensitive to the potentially disturbing effects of environmental stresses, and will build up genotypes which produce the optimum phenotype even under sub-optimal or unusual environmental situations. This type of selection has been referred to as 'canalizing selection', since it brings it about that the epigenetic systems of the animals in the population are canalized, in the sense that they have a more or less strong tendency to develop into adults of the favoured type.

It is well known to all geneticists that different strains differ in their sensitivity to environmental influences. A genetic variability on which canalizing selection could operate must therefore exist. There has, however, been rather little experimental work directly concerned with investigating the response to canalizing selection and the magnitude of the effects which can be produced, although many instances in which canalizing selection was actually practised could probably be found by a careful search of the older literature, particularly that of Plant Genetics.

Among the newer studies on the quantitative genetics of animals, the experiments of Mather (1955) and Tebb & Thoday (1954) perhaps come closest to this aim. They investigated the effects of selection and various other genetic procedures on the difference in the number of sternopleural bristles on the two sides of a Drosophila, and demonstrated that the genotype may have an effect on the magnitude of this difference. It may be presumed that the asymmetry between the two sides of the individual arises in response to slight differences in conditions between the two regions of the developing animal. Nothing, however, is known about the nature of these presumed differences during development. Reeve &

* A preliminary communication of some of these results has been made in *Nature, Lond.*, **183**, 1654 (1959).

Robertson (1954) studied a situation which is perhaps of a similar kind; namely, the differences in the bristle numbers on adjacent abdominal segments in *Drosophila*. They have spoken of these differences as arising from 'intangible accidents of development'. It is, indeed, not entirely clear that either type of difference has any definite ascertainable cause. It is possible that some developmental processes have an inherently stochastic character, and the divergences between the various elements of an essentially repetitive or symmetrical system may probably best be compared to 'noise', in the information-theory sense (Waddington, 1957). It is at any rate not at all clear that the genetic control of such a character can be taken as a guide to the nature of the genetic effects exerted on responsiveness to specific environmental stresses.

Falconer (1957) and Falconer & Robertson (1956) have also carried out experiments which involved selection of a type closely connected with canalizing selection. The former, working on *Drosophila*, selected those individuals whose bristle number on two abdominal segments lay closest to the mean of the population. Falconer & Robertson applied selection in mice for individuals whose weights were closest to the mean. In both these experiments the selection would have involved both the normalizing and the canalizing aspects of stabilizing selection, but in neither case was any definite environmental stress applied, and the relative importance of the two types of selection remains unknown. In the event, neither of the experiments revealed any marked response to the selective pressures applied, which, however, were rather small.

For these reasons it has appeared desirable to carry out an experiment designed to show whether canalizing selection can be actually effective in reducing the amount of phenotypic variation produced by a definite and known environmental stress.

MATERIALS AND METHODS

All the experiments were made on *Drosophila melanogaster*. The development of normal wild-type individuals of most stocks of this species is already rather highly canalized, so that the easily applied environmental stresses produce rather little phenotypic divergence from normality. It might therefore be rather difficult for a selection experiment, lasting a comparatively few generations, to produce marked changes in canalization, and in particular increases of it. The experiments were therefore made on mutant stocks in which, as is well known, the phenotype is often much more variable and sensitive to environmental influences than is that of the wild type. Four mutant types have been studied: Bar, dumpy, cubitus-interruptus, aristopedia-Bridges.

1. *Bar*. The number of facets in the eye is markedly reduced, the effect being stronger in the homozygous females than in the hemizygous males. The number of facets produced by a given genotype is known to be greater at a low temperature than at a higher one. The facet numbers can be counted only with some difficulty in the living fly, and estimations of average facet numbers in a population were therefore made by counts on eyes prepared after death. The parents for carrying

on the selection lines were selected on the basis of estimated eye sizes, as judged by inspection with a dissecting binocular.

2. *Dumpy.* This gene produces a shortening of the wing which is more extreme at high temperatures that at low ones. The effect was estimated by measuring the wing length from the base of the wing to the most extreme point of the rounded tip.

3. *Cubitus-interruptus.* The gaps which this gene produces in the posterior longitudinal vein are more extreme at low than at high temperatures. They were estimated in terms of an arbitrary series of grades founded on that used by Stern & Schaeffer (1943) (see Waddington, Graber & Woolf, 1957). In the figures given in this paper the numerical indices attached to the grades are one less than those used in the paper by Waddington, Graber & Woolf; that is to say, the wild type scores as 0 and the most extreme type as 6. These grades are of course quite arbitrary and can be used only for comparative purposes. The stock employed was a multiple recessive stock containing *ci gvl* ey^{R} sv^{n}.

4. *Aristopedia-Bridges.* This is a fairly weak allele of the well-known gene which tends to convert the aristae into leg-like appendages. The strain used was extracted from the F_2 of a cross between the aristopeida stock maintained in the laboratory and an Oregon-R wild type. The effect is more extreme at low than at high temperatures. It was estimated by a series of arbitrary grades, similar to that employed by Waddington & Clayton (1952). Among the more extreme modes of expression there are several in which the size of the aristal organ becomes reduced. In assigning numerical values to the grades these have been given relatively low values, similar to that of phenotypes in which only part of the arista has been converted into a leg-like structure. It is, however, by no means certain that this is the correct way of envisaging the situation. One of the effects of aristopedia is to cause reductions in the leg, and it may be that these reduced aristal organs really represent an extreme grade in the expression of the genic action, in which the arista is first converted into a leg and then reduced. If that were the case, the reduced organ should perhaps have a higher numerical index rather than a lower one corresponding to its size. It is unfortunate that, as it turned out, a considerable proportion of the populations kept at low temperatures had phenotypes within this range. As will be seen later, the selection practised had little effect in altering the average phenotype of these cold-temperature populations, and it may well be that owing to the uncertainties of the grading system very little selection was in fact exerted on them.

Three types of experiments have been carried out. The first two were both varieties of what may be called alternate selection. In these the strain was kept for one or more generations at one temperature, then moved to the other temperature for one or more generations, and then back again to the first set of conditions, and so on alternately. Breeding always involved selecting against the known environmental effect of the temperature. That is to say, if, as in Bar, a low temperature tends to cause an increase in the number of eye facets and a higher temperature a reduction in facet number, one would select from a population

reared at a low temperature those with the smallest facet numbers, and from a population bred at a high temperature one would select those with the largest number of facets. The two experiments carried out according to this plan differed only in that, in the first of them, one generation at a low temperature of 18° C. alternated with two generations at the high temperature of 25° C., while in the second experiment only one generation at the high temperature followed one generation at the low. In a variant of the first type of experiment two generations at 25° were run simultaneously with one at 18°, and individuals from the second 25° generation were then crossed with those from the 18° generation to produce two new selected lines, one of which was again given two generations at 25° while the other had one generation at 18°, when the process was repeated.

A very different and, as it turned out, much more effective type of selection was applied by means of family selection. In this, a series of pair-matings were set up, and from each pair of parents a number of offspring were reared at 25° and a number at 18°. Family averages of the difference between the phenotypes at these two temperatures were then ascertained by measuring a certain number (usually twenty) of the offspring at each temperature. Selection was made on the basis of these family averages, lines being carried on by breeding together sibs from the families which exhibited the least environmental sensitivity and in which the difference between the offspring at the two temperatures was smallest.

RESULTS

1. *Alternate selection*

The results of the experiments carried out with Bar, dumpy and cubitus-interruptus are given in Tables 1 to 3. It will be seen that there was considerable progress in reducing the environmental sensitivity of the Bar stocks. In dumpy and cubitus-interruptus only rather slight progress was made. This result may be partly due to the necessity to use, in these two stocks, a somewhat inadequate system of scoring by arbitrary grades. The imperfections of this system probably reduce the amount of selection that can be exerted, and it probably reflects only very inaccurately the actual situation in the stocks which eventually arise. In spite of this, some slight reduction in environmental sensitivity has been achieved in nearly all cases. The magnitude of the effects, however, is so slight that it could hardly be taken to be sufficient to establish the reality of the process.

2. *Family selection*

Family selection experiments were carried out with Bar and with aristopedia-Bridges. The results are shown in Tables 4, 5 and 6. In both cases very marked reductions in environmental sensitivity have been achieved.

In these experiments selection was carried out on the basis of the average magnitude of the difference between the high- and low-temperature sibling groups. No attention was paid to the absolute magnitudes of the phenotypic indices. In

Table 1. *Bar. Mean facet number per eye (two sides averaged)*

	Generations									
	1	2	3	4	5	6	7	8	9	10
Temperature	25	25	18	25	25	18	25	25	18	25
♂	91	82·6	222·8	100·0	117·7	191·6	91·0	101·0	182·3	90·0
Difference, 18°–25°			140·2	122·8		73·9	100·6		81·3	92·3
♀	46·9	51·8	160·1	66·8	56·7	107·3	61·0	69·3	95·5	58·4
Difference, 18°–25°			108·3	99·3		50·6	46·3		26·2	37·1

	Generations							
	11	12	13	14	15	16	17	18
Temperature	25	18	25	25	18	25	25	18
♂	127·9	183·8	116·0	106·2	196·0	122·8	110·8	204·8
Difference, 18°–25°		55·7	67·8		89·8	73·2		94·0
♀	73·7	102·5	68·0	63·3	103·4	71·0	71·5	102·7
Difference, 18°–25°		28·8	34·5		40·1	32·4		31·2

the case of Bar this has resulted in the production of a relatively temperature-insensitive eye of a size intermediate between that which was characteristic of the low- and high-temperature populations at the beginning of the experiment. Some representative figures on this point are given in Table 5. With aristopedia the result was rather different (Table 6). The reduction in temperature sensitivity has in essence been brought about by increasing the abnormality of the high-temperature population, in which initially the gene was rather weakly expressed. There has been scarcely any accompanying reduction in the expression in the low-temperature group. As was pointed out in the section on methods, this is probably a consequence of the imperfections of the scoring system, which seems likely to be particularly unreliable in discriminating between the various higher grades of expression, so that it seems probable that in fact very little selection of any kind was actually exerted on the low-temperature populations.

It may appear from Table 4 that the response to selection has been greater than the selection-pressure applied, since in many instances the later generations show a smaller average family difference than that found in the family which was selected in the immediately preceding generation. However, the figures given in brackets in that table, which record the difference between the 25° and 18° groups of the families selected, do not tell the whole story, since selection was also practised *within* the families, by selecting individuals which showed little effect of the temperature treatment. It is therefore impossible to give any accurate analysis of the rate of response to the selection-pressure. The bracketed figures are given merely to show that the response cannot be considered a slow one.

Table 2. *Dumpy. Wing lengths (two sides averaged)*

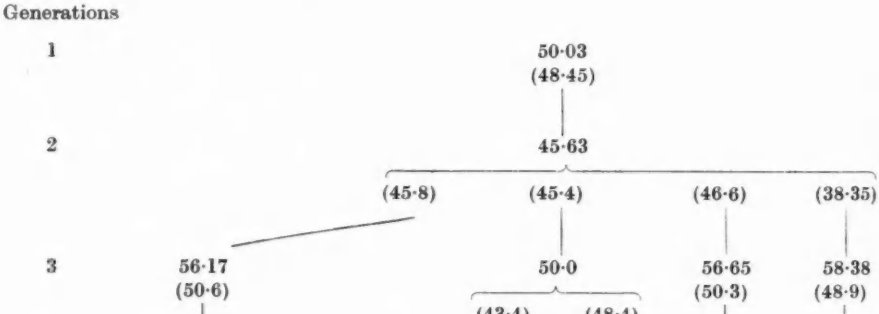
Table 2. *Dumpy. Wing lengths (two sides averaged)*

		Generation											
		1	2	3	4	5	6	7	8	9	10	11	12
Expt. 1	Temperature	25	25	18	25	25	18	25	25	18			
	Wing length ♂	55·8	53·4	70·4	55·3	56·5	76·35	57·65	61	78·4			
	Difference, 18°—25°		17·0	15·1		19·85	18·7		17·4				
	Wing length ♀	58·8	56·95	75·65	59·9	63·3	76·8	62·1	63·1	80·1			
	Difference, 18°—25°		18·7	15·75		13·5	14·7		17·0				
Expt. 2	Temperature	25	18	25	18	25	18	25					
	Wing length ♂	52·8	75·35	58·5	76·15	58·6	77·65	61·45					
	Difference, 18°—25°		22·55	16·85	17·65	17·55	19·05	16·2					
	Wing length ♀	55·05	77·45	61·75	74·45	62·7	78·6	63·95					
	Difference, 18°—25°		22·4	15·7	12·7	11·75	15·9	14·65					
Expt. 3	Temperature	25	25	18	25	25	18	25	25	18	25	25	18
	Wing length ♂	58·1	57·8	76·45	56·1	60·15	75·45	60·2	60·1	76·25	61·5	63·1	79·35
	Difference, 18°—25°		18·65	20·35		15·3	15·25		16·15	14·75		16·25	
	Wing length ♀	59·48	59·4	77·45	60·15	60·85	74·45	59·75	64·0	79·55	64·1	63·5	79·5
	Difference, 18°—25°		18·05	17·3		13·6	14·7		15·55	15·45		16·0	

Table 3. *Cubitus-interruptus. Mean grade (two sides averaged)*

	Generations														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Temperature	25	25	18	25	25	18	25	25	18	25	25	18	25	25	18
♂	138	127	202	151	150	212	140	134	208	140	159	260	156	140	196
Difference, 18°—25°		75	51		62	72		74	68		51	54		56	
♀	93	96	182	98	116	199	127	83	170	113	132	151	133	130	194
Difference, 18°—25°		86	84		83	72		87	57		19	18		64	

Table 4. *Bar. Facet number in females. Pedigree of family selection experiment. The figures give the differences between the means of the family-samples reared at 25° and at 18°. The plain figures give the average of the differences of all families tested; the figures in brackets the difference in the family selected to carry on the line*



In view of the interest which has been taken in asymmetry as an indication of developmental stability, it seemed interesting to inquire whether successful canalization against the effect of temperature was accompanied by a greater stability against the unknown environmental variations to which asymmetry may hypothetically be attributed. The asymmetry of facet numbers was therefore estimated, the measure used being the difference in the logarithms (to base 10) between the larger and the smaller of the two eyes. As can be seen from Table 7,

there was little alteration in the asymmetry during the course of selection; in the twenty-three families measured after selection, the asymmetry at both temperatures was slightly less than it had been in the set of pair-matings from which the

Table 5. *Bar. Facet numbers in females in unselected controls, and in three families resulting from selection*

	No. of Families	Average family mean, at 18° C., and S.E.	Average family mean, at 25° C., and S.E.	Difference, family means, 18° C. and 25° C.
Unselected	9	156·25 ± 4·81	55·46 ± 0·81	100·80 ± 5·42
Selected 6*a*	8	106·02 ± 1·17	91·51 ± 1·67	14·50 ± 2·29
Selected 6*b*	7	100·76 ± 1·38	95·30 ± 1·70	5·46 ± 1·98
Selected 6c	8	111·39 ± 1·72	98·48 ± 2·67	12·92 ± 3·05

Table 6. *Aristopedia-Bridges. Family mean grades*

Generation	No.	18°	25°	Difference
0	10	14·01 ± 0·085	7·16 ± 0·215	6·84 ± 0·177
1	20	13·36 ± 0·130	7·46 ± 0·152	5·89 ± 0·157
2	20	13·74 ± 0·067	7·89 ± 0·141	5·90 ± 0·167
3	20	13·58 ± 0·062	7·39 ± 0·184	6·20 ± 0·20
4	20	13·36 ± 0·07	8·72 ± 0·13	4·65 ± 0·14
5	20	13·60 ± 0·071	10·50 ± 0·179	3·10 ± 0·190
6	19	13·44 ± 0·626	11·13 ± 0·286	2·31 ± 0·268

Table 7. *Mean asymmetry in facet number (log_e scale)*

Selected for	Progeny of	18° C.	25° C.
0 generations	20 pairs	0·102 ± 0·025	0·095 ± 0·018
0 generations	Foundation pair	0·115	0·069
6 generations	23 pairs	0·093 ± 0·026	0·081 ± 0·026

selection started, but the difference is small and non-significant. When one compares the families at the end of selection with the particular foundation pair-mating from which they were all derived, the asymmetry at 25° is found actually to have increased. One may conclude that selection for better buffering against the external temperature has had little or no effect on asymmetry.

DISCUSSION

The family selection experiments, particularly that with Bar, provide a very clear example of the effectiveness of canalizing selection. In the unselected population the eyes of individuals reared at low temperatures have about three times as many facets as those reared at 25°, while by the end of the selection experiments the difference has been reduced to about 10%.

This reduction in environmental sensitivity has occurred in an epigenetic system which is abnormal in the sense that it is affected by an unusual allele, namely, the dominant mutant gene Bar. There seems no reason to doubt, however, that similar processes of selection would be effective in reducing the environmental sensitivity of a population in which no obviously abnormal allele was present. The array of genotypes which fall within the broad category of the wild type all give rise to epigenetic systems which are, in general, rather insensitive to external stresses, but it seems inevitable that amongst them there must be some genotypes which provide better developmental buffering than others. It was not by any means obvious, however, that such an abnormal developmental system as that resulting from the Bar mutant could be brought to a condition of such temperature-insensitivity as we have found. Since canalizing selection, operating through families, has been so extremely effective on this abnormal developmental system after only five generations, one is left with a feeling of considerable confidence as to its powers when acting on more normal populations.

The comparative failure of systems of alternate selection is perhaps not very surprising. When in an 18° population of Bar one selects the individuals with the smallest eyes as parents of the next generation, one is, of course, not only exerting canalizing selection in favour of genotypes which are insensitive to the temperature effect, but one is also applying progressive selection in favour of genotypes which would tend to produce a small number of facets under any environmental circumstances. In the next generation, reared at 25°, selection for large eyes would again favour well-canalized genotypes but would now also involve a progressive selection of the opposite kind to that in the previous generation, since it would favour genotypes which tended to produce large facet numbers under all circumstances. The canalizing selection would only be effective in so far as the two opposite types of progressive selection cancelled each other out, and in proportion to the importance of canalizing factors as opposed to facet-increasing or facet-reducing factors in determining which individuals will be selected. There is no way of telling *a priori* which will be the more important elements in the situation. The fact that in these experiments there was in several cases an immediate slight increase in canalization, but that the rate of progress was not maintained thereafter, suggests that the presence of alleles of different quantitative effectiveness has made it very difficult by this procedure to concentrate alleles whose effect is primarily on environmental sensitivity.

The mathematical theory for selection for canalizing genes has never been worked out. It is, however, easy to show that selection which operates only against the phenotypic effect of a single environment, such as that which was exerted in the alternate selection experiments, would often tend to concentrate quantitatively-acting genes rather than canalizing genes. Consider, for instance, a population which contained a relatively rare allele a whose effect in all environments is to reduce the size of a given phenotypic measure. Let the frequency of a be p. Further, suppose the population contains an allele b with a frequency of p and with the effect that the homozygote bb is less environmentally sensitive. Now,

suppose the population to be in an environment which tends to increase the phenotypic measure, and let selection be applied against this environmental effect. We will suppose that the selected group includes all the genotypes containing *aa*, and that the reduction in phenotypic sensitivity brought about by *b* is such that the genotypes *Aabb* would also fall within it. Then in the next generation

$$p_1 = \frac{2p^2+2pqP^2}{2p^2+4pqP^2} = \frac{p+qP^2}{p+2qP^2}$$

and

$$P_1 = \frac{2p^2(P+PQ)+4pqP^2}{2p^2+4pqP^2} = \frac{pP+2qP^2}{p+2qP^2}.$$

Thus, for the proportional rates of increase of *a* and *b* we shall have

$$\frac{p_1-p}{p} = \frac{q[p+P^2(1-2p)]}{p(p+2qP)} \quad \text{and} \quad \frac{P_1-P}{P} = \frac{2qP(1-P)}{p2qP}.$$

The 'quantitative gene' *a* will increase proportionately faster than *b* if

$$\frac{p+P^2(1-2p)}{p} > 2P(1-P),$$

i.e.

$$P^2 > p(P-Q).$$

It is clear that, with appropriate values of the frequencies, either the quantitatively-acting or the canalizing genes will increase the more rapidly. Without some prior knowledge of the relative frequencies of genes of these two kinds, one cannot predict what the outcome will be.

The lack of response of asymmetry to selection which was effective in reducing sensitivity to the particular environmental variable of temperature is worth noting. But it should occasion no surprise. Reasons have been offered previously (Waddington, 1957) for expecting differences between comparable regions within a single individual body to be controlled by genes which are not the same as those which affect the reaction of the body as a whole to the external environment.

SUMMARY

1. Mutant stocks whose phenotype is affected by temperature were kept at 18° and 25° C., and selection was applied in an attempt to reduce the magnitude of the phenotypic difference caused by the temperatures. The stocks used were Bar, dumpy, cubitus-interruptus and aristopedia-Bridges.

2. When the stocks were kept for one or more generations at one temperature, then transferred for the next generation or two to the other, and so alternately, selection applied against the phenotypic effect of the temperature was only slightly effective in reducing the differences between the high- and low-temperature phenotypes. It is suggested that this was due mainly to the fact that such selection would be expected to operate on quantitatively-acting genes as well as on genes controlling developmental buffering.

3. With family selection, in which the offspring of a pair-mating was divided into two lots, one kept at each temperature, and selection was made on the basis of the differences in family means, progress was rapid. In the unselected Bar stock, the facet number at 18° was initially about three times that at 25°; after six generations of family selection the difference had been reduced to about 10%, the phenotypes at both temperatures being about intermediate between those seen at the beginning of the experiment. With aristopedia the reduction in the difference in family means was also striking, but in this case was achieved by increasing the abnormality of the 25° phenotype, and hardly at all by lowering that of the 18° population. It is pointed out that this is probably the result of an inadequate scoring system.

4. Although the selection in Bar stocks was effective in increasing developmental canalization against external environmental changes (temperature), it had little or no effect on the asymmetry of the facet numbers.

REFERENCES

FALCONER, D. S. (1957). Selection for phenotypic intermediates in *Drosophila*. *J. Genet.* **55**, 551.

FALCONER, D. S. & ROBERTSON, A. (1956). Selection for environmental variability of body size in mice. *Z. indukt. Abstamm. u-VererbLehre*, **87**, 385.

MATHER, K. (1955). Genetical control of stability in development. *Heredity*, **7**, 297.

REEVE, E. C. R. & ROBERTSON, F. W. (1954). Studies in quantitative inheritance. VI. Sternite chaeta number in *Drosophila*; a metameric quantitative character. *Z. indukt. Abstamm. u-VererbLehre*, **86**, 269.

SCHMALHAUSEN, I. I. (1949). *Factors of Evolution*. Blakiston, Philadelphia.

STERN, C. & SHAEFFER, E. W. (1943). On the wild-type isoalleles in *D. melanogaster*. *Proc. nat. Acad. Sci., Wash.*, **29**, 361.

TEBB, G. & THODAY, J. M. (1954). Stability in development and relational balance of X-chromosomes in *D. melanogaster*. *Nature, Lond.*, **174**, 1109.

WADDINGTON, C. H. (1940). *Organisers and Genes*. Cambridge University Press.

WADDINGTON, C. H. (1957). *The Strategy of the Genes*. Allen & Unwin, London.

WADDINGTON, C. H. & CLAYTON, R. M. (1952). A note on some alleles of aristopedia. *J. Genet.* **51**, 123.

WADDINGTON, C. H., GRABER, H. & WOOLF, B. (1957). Isoalleles and the response to selection. *J. Genet.* **55**, 246.

Genet. Res., Camb. (1960), **1**, *pp.* 151–172
With 2 text figures
Printed in Great Britain

Some genetic tests on asymmetry of sternopleural chaeta number in *Drosophila*

By E. C. R. REEVE

Agricultural Research Council Unit of Animal Genetics, Institute of Animal Genetics, Edinburgh, 9

(*Received* 3 *October* 1959)

INTRODUCTION

The variance of a quantitative character in individuals of a single genotype, reared in a given set or range of environmental conditions, is often taken as a measure of the developmental stability of the genotype. Broadly speaking, this variance is the sum of two components: (1) the direct effect of differences betweeen the environments of different individuals—the environmental variance proper, and (2) the effects of local accidents of development, originating perhaps largely at the molecular level, which prevent the perfect replication of the same phenotype even under identical environmental conditions. This variance has been given several names, including 'chance or stochastic variability' (Reeve & Robertson, 1953*b*), 'developmental error' (Clayton *et al.*, 1957), 'developmental noise' (Waddington, Graber & Woolf, 1957), or, when calculated from the uncorrelated variations among two or more elements repeated on the same individual, the 'independent variance'(Reeve & Robertson, 1954). None of these terms is entirely satisfactory, and I shall refer simply to the 'chance variance', except when another term seems more apt.

Whilst it might be supposed that the first, or true environmental, component is generally by far the most important, this is certainly not always the case. Thus Wright (1952), studying the amount of white in the coat of guinea-pigs, concluded that 86% of the total variance of an inbred strain must be attributed to local accidents of development. This proportion was, in fact, the total variance within litters, which could not be due to environmental differences between litter-mates, since there was no correlation between the amounts of white on different regions of the coat of the same animal more than a short distance apart.

Essentially the same approach was used by Reeve & Robertson (1954) in analysing the variation in the number of chaetae on the abdominal sternites of *Drosophila melanogaster* in inbred lines and crosses. Segments 3–5 were counted in males and 3–6 in females, and virtually no correlated variation was found between the numbers of hairs on the different segments, when flies of a single genotype were reared under fairly standard conditions without overcrowding. We concluded that, under these conditions, there was almost no environmental variance proper in hair number, but a large 'chance' component was present, with a coefficient of variation of about 8% per segment, which was probably caused mainly by a lack of precision

in the processes of morphogenesis responsible for chaeta formation. In addition, true environmental variation could be induced by crowding or otherwise varying the environment, and this caused highly correlated variations on the different segments.

This case evidently provides a striking parallel with that of the amount of white in the guinea-pig's coat, referred to above, and very similar results were found for variation in the number of ovarioles in the left and right ovaries of *Drosophila melanogaster* by Robertson (1957). There are typically about twenty ovarioles in each ovary, and crosses between several inbred lines gave a variance per ovary with an average coefficient of variation, for flies reared on the usual fairly standard diet, of about 8%. Approximately 90% of this variance was the result of uncorrelated variations in the two ovaries, and so comes within our category of 'chance' variance. We may note in passing the curious fact that sternites with twenty chaetae and ovaries with twenty ovarioles show almost the same variance due to chance effects, but whether this reflects any fundamental similarity in the causal factors underlying the variation it is not easy to decide.

As a contrast to these cases with the non-genetic variance mainly due to chance effects, wing length in *Drosophila* was found to have an environmental variance of which only about 13% could be attributed to differences in length of the two wings, equivalent to a coefficient of variation of about 0·5% (Reeve & Robertson, 1953*a*). Again, of the environmental variation in body-size, roughly half is due to variations in wing and thorax length which are uncorrelated with each other; this sets an upper limit of about 50% to the chance component for body-size in *D. melanogaster*, reared under the usual standard conditions (Reeve & Robertson, 1953*b*).

The total amount of non-genetic variance is certainly under partial genetic control, since heterozygotes are generally more stable than homozygotes for many quantitative characters, at least in the case of normally outbreeding organisms; and the question naturally arises whether this genetic variation in phenotypic stability affects only the environmental variance proper, or also the chance variance. Reeve & Robertson (1954) found no decrease in the variance of sternite chaeta number in *D. melanogaster*, when inbred lines were intercrossed, and they concluded that the chance variance of this character was not subject to appreciable genetic control. In keeping with this result is the fact that Mr B. J. Harrison selected for twenty generations in an attempt to increase and decrease, respectively, the difference in the number of hairs on the fourth and fifth abdominal sternites, without making any progress (private communication and Harrison, 1954).

As another example, Reeve & Robertson (1953*b*) found virtually no decrease in the uncorrelated fraction of the variance of wing length and thorax length when pure lines were intercrossed, although the correlated variances dropped to less than half.

On the other hand, Mather (1953) crossed two inbred lines of *D. melanogaster*, and found that the mean amount of bilateral asymmetry in the number of sternopleural chaetae was much lower in the F_1 and F_2 than in the parent lines.

Moreover, he was able to raise and lower the amount of asymmetry in a striking manner by selecting from the F_2 of the cross.

We thus appear to have a rather puzzling contrast in the genetic behaviour of two characters, both consisting of the number of chaetae on particular regions of the integument of the same organism, so that we might, on the face of it, expect them to show rather similar responses when inbreds are crossed or when selection is carried out. A number of experiments have been undertaken with the hope of analysing this difference in more detail, and will be described below. These included tests of the amount of genetic variance for sternopleural asymmetry in three wild stocks, and comparison of the changes in variance of the two characters when homozygous lines are intercrossed.

Several wild stocks of *D. melanogaster*, or lines derived from them, were used in these tests, and are listed below with their code letters. They have all been kept in this laboratory in mass culture, for varying periods, and have been shown by various tests to contain plenty of genetic variance for different characters. They are:

Pobla da Lillet	(PdL)	Pacific	
São Paulo	(SP)	Renfrew	(Re)
Crianlarich	(Crian)	Edinburgh	(WE)

GENETIC VARIANCE OF STERNOPLEURAL ASYMMETRY IN WILD STOCKS

The idea put forward by Mather, that the chance variability in sternopleural hair number, as measured by the mean amount of difference between right and left sides, is under genetic control, is a novel one; and it seemed to be of particular interest to test whether there is any appreciable amount of genetic variance for this character in wild stocks. To do this, a progeny test was made on the PdL wild stock, and selection for increased and decreased asymmetry was carried out on the Crian and SP stocks.

For the progeny test, forty pair-matings were made between flies 3–4 days old, chosen at random from the PdL stock. Each pair was allowed to lay eggs for 24 hr. in a 3×1 in. vial containing the usual cornmeal-molasses medium, and then transferred to a fresh vial. Cultures from eggs laid on 3 successive days were raised at 25° C., and the numbers of sternopleural hairs on the two sides were counted on all male and female progeny which survived long enough for the rather laborious work to be completed. All counts were made by the author.

Altogether, counts were made on thirty-one families in males and thirty in females which provided at least two cultures, with an average of 15·3 males and 16·3 females per culture, and 43·5 and 45·1 per family.

The simplest measure of asymmetry is the absolute difference in hair number on the two sides, $|L-R|$ taken regardless of sign, where L and R are the numbers of left and right sternopleurals. This may be compared with the total count $(L+R)$. After fitting constants for day differences because of the variable number of flies per culture, the progeny variances are separated into the mean squares between

days, between families, between cultures within families and within cultures. Since the last two items were in no case significantly different they have been pooled, and the results for both $|L-R|$ and $(L+R)$ are given in Table 1.

Table 1. *Progeny test on sternopleural sums and differences*

	Degrees of freedom		Mean Squares			
			$L+R$		$\|L-R\|$	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
Between days	2	2	55·4*	50·0*	0·32	0·68
Between families	30	29	18·30*	22·52*	1·33*	0·90
Within families	1316	1321	2·72	2·99	0·71	0·81
Heritability %			23·5	25·3	4·0	0·5

* P less than 0·01

Taking first the variance in total count $(L+R)$, we notice a significant difference between days, due to the fact that the third day, with averages of 17·7 in males and 19·1 in females, had 0·6 hairs less than the first 2 days, whose mean counts were almost identical. Both sexes gave a highly significant variance between families, equivalent to a heritability of 23–25% when this is calculated in the usual way, as the ratio of the component of variance between families to the sum of the components within and between families. Clearly there is plenty of genetic variance for total count in the stock.

In the case of asymmetry, there was no day effect, and the variance between families was greater than that within families in both sexes, and significantly so (at the 1% level) in males. This suggests that there is a small amount of genetic variability for asymmetry in the stock. The heritability is estimated as 4% in males and 0·5% in females, and on pooling these figures we estimate the genetic variance as about 2% of the phenotypic variance. We should, therefore, expect to make some slight progress on selecting to increase or decrease the level of asymmetry.

In the two selection experiments, twelve males and twelve females were used as parents each generation. These were mass-mated and their eggs cultured (70 per vial) in vials of the usual cornmeal-molasses medium at 25° C. All the flies emerging in three vials were scored, and the extreme four males and four females from each vial were selected as parents and mass-mated for the next generation. This meant that usually 15 to 20% of the flies examined were selected.

In the first experiment, with the Crianlarich wild stock, one line was selected for the greatest absolute value of $L-R$, and the other for the smallest difference between L and R, regardless of the total number of sternopleurals present. The results are shown to the left in Fig. 1, in which (+) and (−) indicate the lines selected for increased and decreased asymmetry. The top graph shows the changes in mean asymmetry, the middle graph the changes in mean total count, and the bottom graph the changes in the standard deviation of total count.

Both lines were continued for four generations, and moved steadily apart in all three indices. Then the (−) line was discontinued, and four generations later unselected controls (*C*), taken off the mass stock each generation but raised in the same way as the selected lines, were put up concurrently with the (+) line. The experiment was terminated after thirteen generations, and the graphs show the average levels of (+) and controls for each index, over the six generations when they were run together.

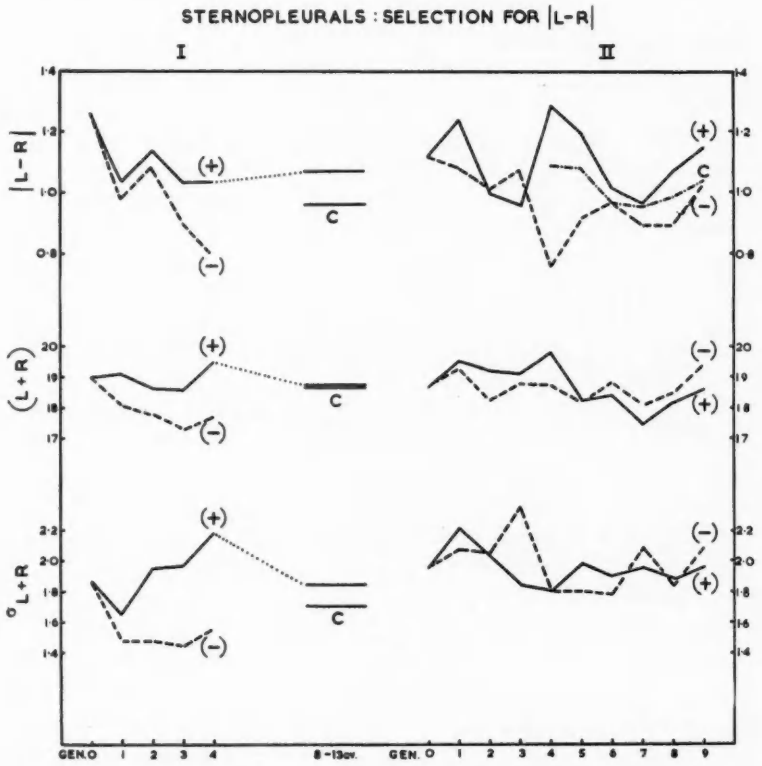


Fig. 1. Selection for increased sternopleural asymmetry (+) and decreased asymmetry (−) in two wild stocks. I: first experiment, on the Crianlarich stock. II: second experiment, on the SP stock. *C* indicates unselected control stock.

The trends in asymmetry are a little difficult to interpret in view of the high level at generation 0, which may possibly have been caused by some undetected environmental factor. Changes in asymmetry were certainly induced by selection, and the impression given by the graph is that the (−) made more progress than the (+) line. The latter had evidently made some progress by the end of the experiment, but there is little doubt that selection for decreased asymmetry had been effective in the (−) line. The changes in asymmetry were accompanied by parallel

changes in total count, leading to a mean difference of nearly 2 hairs at generation 4, but this is hardly sufficient to explain the change in asymmetry as a scale effect. Rather dramatic changes in the same sense also occurred in the standard deviation of total count, so that the (−) line became both less asymmetrical and less variable in total count than the (+) line.

The second experiment was made on the SP wild stock, and this time controls were maintained from the start and were scored each generation. The results of the nine generations of selection in this experiment are shown in the right-hand graphs of Fig. 1.

In the first three generations little progress was apparent, since the (+) line was no more asymmetrical than the (−) line, but from generation 4 onwards $|L-R|$ was always greater in the (+) line, although the deviation between the two lines was never as large afterwards as it was at generation 4. A transient effect, either genetic or environmental, must have been responsible for this trend. The line of the controls (C) is only plotted from generation 4 onwards, and clearly runs in between the two selected lines. This means that selection was able both to increase and decrease the amount of asymmetry in the parent wild stock, and supports our tentative conclusion from the previous experiment.

The two lower graphs indicate that, in this experiment, the changes in asymmetry produced no correlated changes in total count or its variance. The (+) line had a slightly larger total count than the (−) line up to generation 5 and a smaller count afterwards, while no definite trend appears in its standard deviation. The control line is not plotted for these indices, since its values intermingle with those of the two selected lines in both cases.

Both selection experiments, then, give a clear indication that the level of bilateral asymmetry of the sternopleurals can be both increased and decreased in a wild stock by selection, and these changes are not necessarily accompanied by change in the total count or its variance.

Each selection experiment can be made to provide a rough estimate of the genetic variance in the parent wild stock, calculated as the fraction of the total selection pressure applied which is converted into genetic progress. This fraction is known, perhaps a little ambiguously, as the 'realized heritability' (Falconer, 1954), and has the practical disadvantage that it often varies a good deal with the number of generations of selection included. Where there are rather marked fluctuations from generation to generation in total progress, due possibly to evanescent environmental effects, a good compromise is to assume a linear rate of progress, so that the mean of all the differences between the two lines—or between one line and its control—can be taken as the result of half the overall selection pressure applied.

We have applied this method to the first four generations of selection in the first experiment and the full nine generations in the second. In the latter, the progress of each line is compared with the controls. The results are summarized, and compared with the corresponding figure from the progeny test, in Table 2.

The progeny test gave a figure of about 2%, while the estimates of heritability obtained from the two selection experiments are 2·5% for the Crian and 1·3% for the SP lines. The last figure is the average of 1·6% for (+) selection and 1·0% for (−) selection. In general, therefore, the estimates from the three stocks are in

Table 2. *Heritability of sternopleural asymmetry*

	Average $\lvert L-R \rvert$ per generation		
Selection in:	Selection differential	Advance	Heritability %
SP Stock (+)	1·33	0·021	1·58
(−)	0·97	0·010	1·05
Total	2·30	0·031	1·32
Crian stock: total	2·46	0·062	2·52
Progeny test on PdL stock			1·98

remarkably good agreement, and suggest that in typical wild stocks some 2–3% of the phenotypic variance in asymmetry is genetic.

It is of interest to compare our rates of progress with those obtained by Mather (1953) on selecting from the F_2 of a cross between two inbred lines. Mather measured asymmetry by the mean square difference, $V = (L-R)^2/n$, where n is the number of flies scored; and Table 3 gives V for the first five generations in our two selection experiments.

Table 3. *Mean square differences in selection experiments*

	First experiment on Crian			Second experiment on SP		
	(+)	(−)	Divergence	(+)	(−)	Divergence
Gen. 0	2·64	2·64	0·0	2·14	2·14	0·0
1	1·86	1·63	0·23	2·50	1·94	0·56
2	2·04	1·92	0·12	1·87	1·73	0·14
3	1·74	1·50	0·24	1·63	1·91	−0·28
4	1·78	1·14	0·64	2·35	1·05	1·30
5	1·76	—	—	2·37	1·59	0·78

On selecting the extreme 10% each generation, Mather was able in a few generations to establish a (−) line with V about 1·0 and a (+) line with V 2·5 or more, so that the total divergence in V was at least 1·5 units. Our own progress, selecting the extreme 15–20% of the population, appears to have been rather less dramatic than this, since V in the (+) and (−) lines only diverged by 0·6 unit after four generations of selection in the Crian stock, and fell away again after reaching a divergence of 1·3 units at generation 4 in the SP stock. This suggests that the F_2 of the two inbred lines crossed by Mather had rather more genetic variance

in asymmetry, in a form readily available for selection, than the two wild stocks.

A particularly important point is that both series of selection experiments are consistent in demonstrating that asymmetry can be reduced well below the level of wild stocks or heterozygous genotypes by direct selection. This point will be discussed later.

One difficulty in interpreting the graphs needs further comment: mean asymmetry shows striking fluctuations from one generation to the next, in both experiments. In the first test, for example, $|L-R|$ starts at 1·26 and falls to 1·03 and 0·98 in the (+) and (−) lines in the next generation, although the total count remains almost constant. This drop is as large as the final divergence between the two lines of 0·25 unit. The parallel fluctuations of the two lines in this experiment strongly suggest the effect of some environmental factor, changing from one generation to another; but it must be admitted that to accept this hypothesis blurs the distinction between environmental and 'chance' variability, since it suggests that the latter can be strikingly modified by undetected changes in culture conditions, which we attempted to keep constant. It is, perhaps, just possible that the change between generations 0 and 1 reflects a change in genetic equilibrium when the stock was transferred from bottle culture to vials.

Equally striking fluctuations, though not always parallel, occurred in the second experiment, particularly over generations 4–6. Possibly one of the genes specifically affecting bristle variance, which turned up in the SP inbred lines to be described later, and so were probably also present in the wild stock, contributed to some of these fluctuations, but we have no further evidence on this point. What is clear, however, is that the changes in absolute level of asymmetry from one generation to the next cannot be taken as a direct measure of the effects of selection, and we must base our conclusions on comparisons between lines reared together.

In the next section we shall examine the behaviour of the sternopleural variances in crosses between inbred or otherwise homozygous lines, in comparison with the variances of the sternite counts.

THE EFFECTS OF INTERCROSSING HOMOZYGOUS LINES

As we noted earlier, published data suggest that the variances of sternopleural and sternite chaeta number behave differently when inbred lines are intercrossed; but a larger body of evidence is needed on this point, since the former character was only examined in a single cross. Further data have now been collected, and their analysis will be summarized below.

A number of lines were available which had been prepared by introducing third chromosomes, picked at random from the Renfrew wild stock, in homozygous phase into an unrelated inbred line (WE 3), in such a way that the other chromosomes of this line remained homozygous. These lines were prepared for another purpose, and their method of preparation will be described elsewhere. Although

they differed only in their third chromosomes, there were marked differences among them in such characters as body-size and the numbers of sternite and sternopleural hairs.

Eight of the lines which showed no signs of visible mutations on their third chromosomes were selected and cultured at the same time as eight intercrosses between them, eggs being cultured on the usual medium at 25° C., with seventy eggs per vial so as to avoid overcrowding. Thorax length was measured on forty females, while the sternopleurals and the hairs on sternites 3 to 5 were counted on thirty males of each mating. The behaviour of the mean and variance of each character, averaged over the heterozygotes and over their mid-parents, is given in Table 4, together with the average percentage change from homozygote to heterozygote.

For the sternopleurals, we have their sum (s), and its variance (σ_s^2), and the mean asymmetry $d = |L - R|$ and mean square asymmetry $Av(d^2)$, calculated as the mean value of d^2. This is identical with Mather's V.

In the case of the abdominal sternite hairs, we have the sum of segments 3 to 5 (s), the variance of this sum (σ_s^2), and the sum of the independent variances on the three segments (σ_d^2), which is calculated so as to be directly comparable with the mean square asymmetry of the sternopleurals. For this purpose we suppose that the variance of each segment is made up of a fraction common to all segments (σ_c^2) and an independent fraction (σ_i^2), and that these fractions are the same for each segment. The two components are then estimated from the observed variances as follows (cf. Reeve & Robertson, 1954):

$$V(\Sigma) = 9\sigma_c^2 + 3\sigma_i^2, \qquad V(\Sigma) - \Sigma(V) = 6\sigma_c^2,$$
$$\Sigma(V) = 3\sigma_c^2 + 3\sigma_i^2, \qquad 3\Sigma(V) - V(\Sigma) = 6\sigma_i^2,$$

where $V(\Sigma)$ is the variance of the sum of the counts on the three segments, and $\Sigma(V)$ is the sum of the separate segment variances. Our index σ_d^2 is then calculated as $\frac{1}{2}[3\Sigma(V) - V(\Sigma)]$, and provides an estimate of $3\sigma_i^2$, or the sum of the independent variances of the three segments.

Applying this method to the sternopleurals, it is easy to see that

$$\sigma_s^2 = 4\sigma_c^2 + 2\sigma_i^2,$$
$$Av(d^2) = 2\sigma_i^2.$$

It will be noted that the mean square asymmetry measures the amount of independent variance in the two groups of sternopleurals, and in the same way σ_d^2 for the sternites may be taken as a measure of the amount of antero-posterior asymmetry among the sternites, after adjustment for differences in their mean counts.

Finally, we have the mean and variance of thorax length. Thorax length shows the familiar result that mean size is about 2% larger and the variance about 32% less in the heterozygotes than in the parent lines. The total sternopleural count is a little lower (2·6%) and its variance is 7·5% higher in the crosses, though

neither of these changes is statistically significant. But both the mean asymmetry ($-13{\cdot}5\%$) and the mean square asymmetry ($-18{\cdot}0\%$) are significantly reduced in the crosses, and the latter figure is remarkably close to the $17{\cdot}4\%$ decline in mean square asymmetry obtained by Mather (loc. cit.) on crossing an Oregon and a Samarkand inbred line together. His figures are given in the furthest right-hand column of Table 4.

Table 4. *Average indices of eight homozygous lines and eight intercrosses (Re in WE 3)*

	Sternopleurals				
	s	σ_s^2	d	$Av(d^2)$	$Av(d^2)$ (Mather)
Homozygotes	17·46	1·45	1·108	1·99	2·22
Heterozygotes	17·00	1·56	0·958	1·63	1·84
Per cent change	−2·6	+7·5	−13·5	−18·0	−17·4

	Abdominal sternites 3–5			Thorax length (1/100 mm.)	
	s	σ_s^2	σ_d^2	Mean	σ^2
Homozygotes	59·38	8·70	7·24	105·0	2·09
Heterozygotes	59·21	8·76	7·68	107·1	1·43
Per cent change	−0·3	+0·7	+6·1	+2·1	−31·6

In contrast to these results, the number of hairs on the abdominal sternites shows no appreciable change in mean or variance ($-0{\cdot}3$ and $+0{\cdot}7\%$ respectively), while the independent variance is higher, if anything, in the crosses than in the parent lines, the net change being $+6\%$. Clearly in this set of crosses the amount of bilateral asymmetry of the sternopleurals was substantially reduced, but no parallel reduction occurred in the corresponding antero-posterior asymmetry between adjacent sternite counts, so that the difference in behaviour of the two groups of bristles seems well established.

A further interesting point is that the variance of total count is less than the mean square difference for the sternopleurals, at least in the homozygotes, so that the variance common to the two sides (σ_c^2) is negative, or, in other words, there is a negative correlation between the two sides. This is about $-0{\cdot}15$ in the homozygotes, but virtually disappears in the crosses ($-0{\cdot}02$), because of the reduction in mean square difference.

These results, taken together with the data published previously, seem perfectly clear, but some further tests, made on another set of homozygous lines and on a set of inbred lines, obscured the clarity of the picture because of the presence of what appeared to be specific genes affecting bristle number and variance in the lines tested.

First, a set of homozygous lines similar to those discussed above was created by making third chromosomes of the SP wild stock homozygous in the genetic background of the WE 3 inbred line. Of the eight lines fully tested, four were found to be homozygous for an allele of the third-chromosome recessive gene polychaetoid (*pyd*). Yet more surprising, the other four lines all carried another third-chromosome recessive effect, which will be referred to as 'sternopleural gaps' (*stg*). This was probably a single gene effect, though not established as such, and caused gaps to appear in the row of sternopleural hairs on one or both sides, at least partly by replacing individual hairs by sockets without hairs.

The means and variances of sternopleural and sternite counts for the two groups of lines and some intercrosses are shown in Table 5, taken from a test made in the same way as before. In the top line we have the average indices of the eight Renfrew lines from Table 4, for comparison. Below are the averages for the four polychaetoid lines and the four lines showing sternopleural gaps.

Table 5. *Characteristics of* (*SP in WE 3*) *lines compared with* (*Re in WE 3*) *lines*

	Sternopleurals			Sternite counts		
	s	σ_s^2	$Av(d^2)$	s	σ_s^2	σ_d^2
Re in WE 3 averages (8 lines)	17·5	1·45	1·99	59·4	8·7	7·2
SP line averages						
4 *pyd* lines	20·8	2·92	2·78	60·3	13·4	11·7
4 *stg* lines	17·0	3·96	3·80	55·8	12·1	7·0
5 crosses	17·3	2·09	1·71	59·2	10·2	8·4
Per cent change	−8	−39	−48	+2	−20	−11

The *pyd* lines had, on the average, about four more sternopleural and four more sternite hairs than the *stg* lines, but the latter had some 30% higher sternopleural variances (3·96 against 2·92 for σ_s^2, and 3·80 compared with 2·78 for $Av(d^2)$). Both series had much higher variances than the corresponding Renfrew-line averages of 1·45 and 1·99. Both SP series also had increased variances for total sternite count (13·4 and 12·1 compared with 8·7), while the *pyd* lines had an increased independent variance for the sternite hairs. Thus in general the counts were more variable and the asymmetry greater in the SP lines.

A set of five crosses between SP lines such that *pyd* or *stg* or both were heterozygous but neither was homozygous are summarized in the fourth line of the table, and the percentage changes in their indices, compared with the average of the *pyd* and *stg* lines, are given in the last line. These crosses agree remarkably well in their indices with the crosses between the Renfrew lines of Table 4, so evidently neither gene has an appreciable effect on variance or asymmetry when heterozygous. The result is that the crosses show reductions of some 40–50% below the parent values in the variance and mean square asymmetry of the sternopleurals, while less drastic but still appreciable reductions occur in the corresponding sternite variances.

Some crosses were also made between SP lines carrying the same gene, and these had just as much variance and asymmetry as the parent lines, suggesting that the increased variances in these lines were due to the *pyd* and *stg* genes themselves, rather than to the overall homozygosity of the third chromosome. It is also of interest that one of the 'sternopleural gap' lines had four flies, out of the thirty examined, with differences of 5, 6, 7 and 8 hairs between the two sides, against a mean sternopleural count of 8·6 hairs per side. The resulting mean asymmetry was 2·13 and the mean square asymmetry 8·7, values enormously greater than have been recorded before.

In a final test, four inbred lines from the Pacific wild stock and six intercrosses were cultured in the usual way, and forty to eighty flies of each sex per mating were scored for sternopleurals, the variable numbers arising because of variable fertility in the different lines. In addition, samples of each line and cross were reared in sterile cultures on 50% concentration of synthetic medium C of Sang (1956). This reduced diet has the effect of reducing body-size (thorax length) by about 12%. The cultures of this test were all prepared by my colleague Dr F. W. Robertson for other purposes, and I am indebted to him for making the flies available to me. Males only from the synthetic-medium cultures were scored for sternopleurals.

The test on the full diet is analysed in Table 6, where the two sexes are treated separately. In males, the results differ strikingly from the earlier tests (Tables 4

Table 6. *Sternopleural indices in Pacific inbreds and F_1's: normal diet*

Averages of six crosses among four inbred lines

Males	s	σ_s^2	d	$Av(d^2)$
Mid-parent mean	18·54	4·79	1·216	2·71
F_1 mean	17·53	3·70	1·313	2·86
Per cent change	−5·4	−22·7*	+8·0	+5·6
Females				
Mid-parent mean	18·18	3·88	1·378	3·03
F_1 mean	17·78	2·80	1·230	2·61
Per cent change	−2·2	−2·1	−10·7†	−14·0

* P less than 0·001. † P less than 0·05.

and 5), since the F_1's show a sharp drop of 22·7% in the variance of total count, and yet have actually an increase in mean asymmetry (d) which is almost statistically significant. The females, on the other hand, behave much as the Renfrew series of Table 4, since the level of asymmetry declines significantly while there is little or no change in total count or its variance. So the curious fact emerges that the males show an increase and the females a decrease in sternopleural asymmetry, when the Pacific inbred lines are intercrossed.

It is possible that here again we have the intervention of one or more genes with specific effects on bristle number and variance, since a variable tendency was noticed for certain bristle abnormalities to occur in the four Pacific inbred lines used and in the intercrosses between them. These took the form of missing dorso-central or scutellar bristles, the bristle being represented only by its socket, or of extra scutellar bristles. As an example, in one of the inbred lines, twenty-seven females possessed, between them, 1 extra scutellar bristle, 34 scutellar sockets without bristles, and 43 missing dorso-central bristles.

These abnormalities occurred with varying frequency, usually higher in females than in males, in all the intercrosses, and also in the Pacific wild stock, so it appears that a genetic condition present in the wild stock was fixed in all the four inbred lines. This is particularly surprising, since the wild stock originated from a large sample of wild flies and has been kept in a large mass culture since. Further genetic analysis is obviously desirable, but is likely to be difficult because of the variable and rather low frequency of manifestation of the effect.

The point of main interest in the present context is that one or more genes affecting the regularity of the bristle patterns are present in the inbred lines of the Pacific stock as in the SP lines, and that in both series they appear to cause a greatly increased variance of total sternopleural count, and also an increased asymmetry as measured by $Av(d^2)$. Intercrosses which leave the genes responsible for these effects homozygous produce little or no reduction in variance and asymmetry.

Some sternopleural indices for the different series are gathered together in Table 7, and compared with those of the PdL wild stock, obtained from the

Table 7. *Sternopleural indices in different lines and crosses*

	s	σ_s^2	$Av(d^2)$ $=2\sigma_i^2$	$2\sigma_e^2$	r_{LR}
Renfrew lines (males)					
Homozygotes	17·5	1·45	1·99	(−0·27)	−0·16
Heterozygotes	17·0	1·56	1·63	(−0·04)	−0·02
SP lines (males)					
Pyd	20·8	2·92	2·78	0·07	+0·02
Stg	17·0	3·96	3·80	0·08	+0·02
Intercrosses	17·3	2·09	1·71	0·19	+0·10
Pacific lines					
Inbred males	18·5	4·79	2·71	1·04	+0·28
F_1 males	17·5	3·70	2·86	0·42	+0·13
Inbred females	18·2	3·88	3·03	0·42	+0·12
F_1 females	17·8	3·80	2·61	0·60	+0·19
PdL wild stock					
Males	18·1	2·72	1·69	0·52	+0·23
Females	19·5	2·99	1·84	0·58	+0·24

progeny test. Besides the total count (s) and its variance, and the mean square asymmetry (which also estimates $2\sigma_i^2$), we give the corresponding variance due to correlated variations on the two sides ($2\sigma_c^2$) and the correlation between the two sides, which may be estimated as $\sigma_c^2/(\sigma_i^2+\sigma_c^2)$.

The mean count is much the same in all series, apart from the three to four extra bristles in the *pyd* lines, and we do not need to consider possible corrections for scale effects. The asymmetry variance is very similar in the Renfrew crosses (1·63), SP crosses (1·71) and wild-stock males (1·69), and these figures probably represent the general level for heterozygotes with seventeen to eighteen bristles. Nevertheless, selection is apparently able to reduce the mean square asymmetry to not much above unity, without changing total count. The level of asymmetry is much higher in the Pacific lines and crosses and in the SP lines, probably because of the homozygosity of specific genes which affect the bristles and not because of the general level of homozygosity.

From the fourth column of Table 7, we see that there is a large correlated variance in the Pacific lines and crosses, but not in the SP lines, so that the fixation of particular genes affecting bristle variability may cause correlated or random fluctuations in count on the two sides. These variations are, of course, non-genetic in origin, but it is not at present clear whether they all come within our category of 'chance' variation or whether the correlated variations are the result of the Pacific-line genotypes possessing a greater sensitivity to environmental effects. What does stand out, however, is that the magnitudes of the non-genetic variances and their behaviour on crossing are very strikingly influenced by the particular lines we select.

The correlation coefficients listed in the last column of Table 7 show that a non-genetic correlation, as in the Pacific-line males, may be as high as the correlation in the wild stock, which one would suppose was entirely genetic. Evidently it would be unwise to assume that the correlated and uncorrelated fractions of the variance in total sternopleural count will necessarily divide it into the genetic and non-genetic fractions.

As a final point, Table 8 shows the effect of restricting the food intake, by using a diluted synthetic diet, on the various sternopleural indices. Data are only available for the Pacific inbred and F_1 males, and in view of their peculiarities it cannot be assumed that other lines would behave in the same way. The table gives the average mid-parent and F_1 values on normal and restricted diet, and the percentage reduction on the reduced diet, for four crosses among the four inbred lines.

Body surface area, measured by the square of thorax length, was reduced in both series by about 23%, but the other indices responded rather differently in inbreds and crosses. In fact, it appears that the total count and its variance were reduced most in the inbreds, while the two measures of asymmetry came down most in the crosses. This result is rather suggestive, and could, no doubt, be fitted in with some recent speculative deductions about the advantages in combined phenotypic stability and flexibility of heterozygotes compared with homozygotes.

Table 8. *Effect of diet on sternopleural indices*

	Pacific inbred lines and crosses: males Sternopleural indices				Thorax area
	s	σ_s^2	d	$Av(d^2)$	
Full diet					
Mid-parent	19·34	5·08	1·24	2·85	
F_1	18·38	3·78	1·34	2·93	
Restricted diet					
Mid-parent	15·75	3·78	1·15	2·34	
F_1	15·98	3·64	1·12	2·12	
Per cent reduction					
Mid-parent	18·5	25·6	7·0	17·9	23·4
F_1	13·1	4·0	16·3	27·4	22·7

Thus it might be argued that the greater reduction in asymmetry shown by the F_1's reflects their greater developmental flexibility, while their smaller change in total count and its variance reflect their greater developmental stability, compared with the inbred lines, both being aspects of the greater developmental homeostasis of the heterozygotes. Unfortunately, the argument can be inverted with equal effect, and a satisfactory interpretation must wait until we have a broader experimental and speculative basis to build on.

DISCUSSION

In discussing experiments on quantitative characters, there is a tendency, illustrated in the last paragraph, for theoretical geneticists to jump from general deductions based on evolutionary suppositions to the interpretation of the statistical indices in terms of which the behaviour of the character is expressed, without giving much attention to the possible biological attributes of the character concerned. Unfortunately these statistical parameters have a very limited power of self-expression, so that whatever theoretical constructs the experimenter starts with may easily be found to have been proved by the experimental behaviour of the parameters he calculates. Our difficulty is, of course, to determine what are the significant biological attributes of any character, from the genetical point of view, or to express them in any but rather general and ambiguous terms. Thus it is an acceptable tautology that natural selection favours maximum 'fitness', of individual or population, but very difficult to lay down what is the relation of any quantitative character to fitness. Even when it seems obvious that more of some character such as egg production is generally of selective advantage, we are far from disentangling the complex interplay of genetical and physiological correlations which obscure any simple relationship between the two.

In the case of the two bristle-count characters, our limits of plausible deduction are yet more restricted, since even their main functions are quite uncertain.

Thoday (1958) states that the number of sternopleural chaetae must have 'adaptive significance', since different populations and species have different characteristic numbers, so that the character is affected by natural selection; but though the fact that different populations have different means must be the result of natural selection (if genetic drift is not responsible), it does not follow that these differences must have any adaptive significance in terms of the character concerned. Many genes are known to act pleiotropically, and non-adaptive changes in one character may be caused by selective forces acting on another, and causing changes in the frequencies of genes which have pleiotropic effects on both. The sternopleurals are most unlikely to have a special set of genes to themselves, with no other effects, and some of the genes which influence their number probably have also more important effects on the integument, or other tissues or organs, which need to change adaptively with change of environment. This, or mutal adjustment of gene frequencies in different genetic backgrounds, is quite sufficient to explain differences in mean count of different populations.

It still remains an open qusetion, then, whether the number of sternopleurals is an adaptive character in the sense that it matters to the individual or population whether it has one or two bristles more or less on each side, or in the sense that the optimum number of bristles is different in different environments. It is worth while looking at these bristles to see what their appearance suggests. There are, in fact, three very stable larger bristles forming a triangle at the dorsal end of the group, and a variable row of smaller ones extending ventrally, together with a sporadic occurrence of very small hairs near the large dorsal bristles. The group occupies a triangular region of hard sclerotized integument near the base of the second leg, and is surrounded by seemingly equally well sclerotized integument without bristles.

Now, if any of these bristles have a specific function, it is certain to be the larger and more stable ones at the dorsal end of the group; and it is quite likely that the smaller hairs are simply a by-product of the morphogenetic forces required to create the three larger bristles. If this is the case, their number would depend on the balance between forces which have other primary functions in development. Selection to alter the number of sternopleurals would eventually disturb this balance, with consequent effects on other features of development. These suggestions are, of course, speculative, and they are put forward to draw attention to a neglected aspect of the whole problem and to emphasize the danger of attaching labels with an evolutionary flavour to characters whose whole biological basis is unexplored and has been left out of account. The alternative hypothesis, that it is of adaptive significance to the fly to have one or two more or less of these small hairs, is very difficult for anyone who has gazed at them for long to believe.

A different aspect was considered by Mather (1953), who pointed out the interesting possibilities of bilateral asymmetry as a subject for genetic study. In particular, it provides a means of investigating the genotypic stabilization of developmental processes, since the two sides of an individual develop under the influence of identical sets of genes, so that differences between them must be due

to what he calls 'local upsets' in development. The level of asymmetry must reflect the degree to which the individual or genotype is able to correct such local accidents, and so measures its developmental stability. Mather chose sternopleural asymmetry because of its ease of study, and Thoday (1958) also takes up this point and argues that this character is 'a useful measure of developmental homeostasis'.

The argument implied here is the familiar one that adapted populations possess genotypes which are better 'canalized in development' (Waddington, 1953), or have more 'developmental homeostatis' (Lerner, 1954), whichever term we prefer, because of natural selection, and this makes them better able to resist the effects of environmental variables or accidents of development in establishing a constant phenotype, than are less well-adapted genotypes. Likewise, heterozygotes will have the same advantages over homozygotes in outbreeding species. The further assumption is clearly implicit in the statements of Mather and Thoday that this greater developmental stability will affect all characters, so that any bilateral character we like to study will give a measure of the same stabilizing forces.

This is, of course, a most engaging thesis, and suggests a method of getting at selective effects which cannot otherwise be investigated except in clones of identical genotype, such as inbred lines and crosses between them. Unfortunately, the experimental results so far obtained do not lend the thesis much support. Mather (1953), for instance, found a higher asymmetry in two inbred lines than in the F_1 and F_2, but selection from the F_2 reduced the level much further still; and my own selection experiments, described earlier in this paper, also show that asymmetry can be reduced well below that of a wild stock by a few generations of selection. Now one must conclude either that the (−) selected lines in these experiments have a much greater developmental homeostasis than the wild stocks or F_2 from which they originated, or that the level of asymmetry is not a measure of developmental homeostasis. The first of these conclusions defies common sense—a wild stock must surely be maintained at or near the maximum level of homeostasis by natural selection—and we are forced to conclude that the level of sternopleural asymmetry does not give us an adequate index of homeostasis, in so far as this is a general characteristic of the organism.

Crosses between inbred lines or artificially created homozygotes usually produce a reduction in asymmetry, so that the character often behaves in this respect like so many other quantitative characters in different species. This decline may, in fact, be the result of increased general developmental stability in heterozygotes; but it must be borne in mind that this is a very crude comparison, since homozygotes from an outbreeding species are genetical oddities, which would have no chance of surviving in competition with normal individuals. A good correlation between the length of chromosome homozygous and the non-genetic variance has been found for body-size and rate of egg-production in *Drosophila*, by Robertson & Reeve (1955), but it has yet to be seen whether any vestige of such a correlation occurs for asymmetry. The fact that there are very different levels of asymmetry in different homozygotes and that the level is not always reduced on crossing,

suggests that any such correlation which occurs will tend to be swamped by other effects.

In spite of these uncertainties of interpretation, it would be of interest to follow changes in asymmetry in populations subjected to various experimental treatments, such as inbreeding or selection for some character. Thoday (1958) studied the behaviour of the index: mean asymmetry divided by total count (d/s in our terminology), when total count was changed by selection, and found that his index increased with selection in both directions. This suggested the occurrence of a phenomenon which one could predict on theoretical grounds—a deterioration in homeostasis with selection. However, an awkward question of scale arises here.

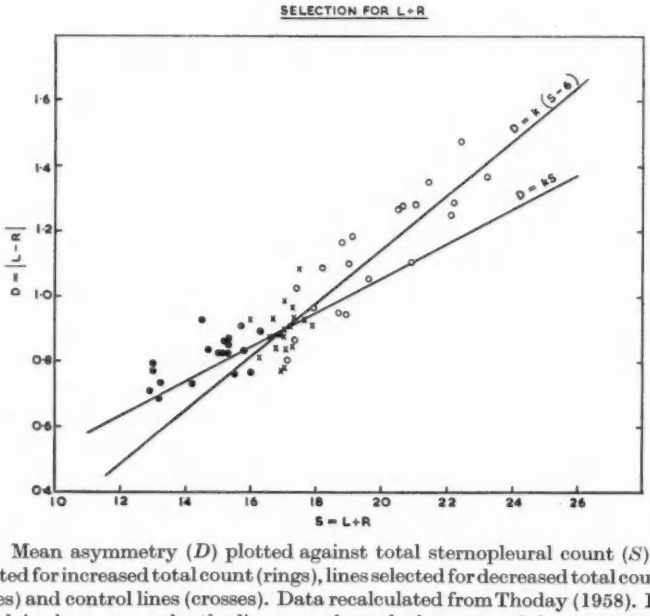


Fig. 2. Mean asymmetry (D) plotted against total sternopleural count (S) in lines selected for increased total count (rings), lines selected for decreased total count (solid circles) and control lines (crosses). Data recalculated from Thoday (1958). For each line, k is chosen to make the line pass through the mean of the control points.

Change in mean count will automatically produce a change in mean left-right asymmetry, quite apart from any change in developmental stability, and the problem is how to eliminate this scalar effect in studying the effects of selection. Thoday's choice of the index d/s is obviously unsatisfactory, since variation in count and asymmetry will depend entirely on the variation in the smaller bristles, at least in the earlier generations of selection. A much better index would be $d/(s-6)$, the denominator being the total count excluding the three stable bristles on each side.

Thoday's data on selection for high count (rings), selection for low count (solid circles) and controls (crosses) have been replotted in the form D against S, in Fig. 2, where $D = |L-R|$ and $S = (L+R)$. The figures have actually been recalculated from his Table 3 and Fig. 2, and may be taken as accurate enough for our purpose.

Two lines have been drawn on the graph, both through the mean of the points for the controls. The line $D = kS$ gives the expected scalar change assuming Thoday's index D/S is the correct one, and we see that the majority of points for both up and down selection have values of D above this line. This leads to Thoday's conclusion that homeostasis deteriorated in both selection lines.

The second line is $D = k(S-6)$, and gives the expected scalar change with our more realistic index $D/(S-6)$. This line runs well below the down selection line, but runs through the middle of the points from selection for increased total count, so that with this scale we should conclude that selection for reduced count caused a rapid deterioration in homeostasis, while selection for increased count left it virtually unaffected.

No doubt we could argue, plausibly enough, that any selection which alters the co-adapted gene pool of the wild stock is bound to reduce the level of homeostasis; but since we have been forced to conclude that mean asymmetry tells us very little about the level of homeostasis, it follows that Thoday's experiment gives us no information about the effects of selection on homeostasis. Nevertheless, the scalar problem is an important one, and Thoday's approach provides a good illustration of the dangers pointed out in the first paragraph of this discussion.

Something must finally be said about the differences in behaviour of the sternopleural and sternite characters. Our results are in agreement with the previous conclusion that the variances in number of sternite bristles—both the total variance and the independent component—hardly change when homozygotes are intercrossed, with the exception of the lines homozygous for the gene *polychaetoid*, which has specific effects on the various bristle regions. Thus no question of developmental stability seems to arise here, since the homozygotes are no less stable than heterozygotes. But, in examining the two characters, sternite and sternopleural bristles, certain important differences become evident which might account for their difference in genetic behaviour.

First, as regards origin, the sternopleurals on each side are believed to develop from part of a single imaginal disc, and the discs on the two sides are certainly derived from groups of cells which separate from each other at some time during embryonic development, since the rudiments of each disc can be detected in the newly hatched larva (Auerbach, 1936). Hence separation of the cell lineages responsible for each group of sternopleurals must occur extremely early in development. Each sternite, on the other hand, is the product of two cell groups, the hypodermal histoblasts, one on each side, so that it is actually of bilateral origin. These histoblasts can first be detected in the early pupa (see discussion in Reeve & Robertson, 1954), and those on one side which contribute to the sternites of the different segments probably only separate from each other late in larval development. The bilateral origin of each sternite would eliminate any asymmetry of the type which affects the sternopleurals, and the comparatively late separation of the elements giving rise to the different sternites might limit the influence of different genotypes on the level of stability.

Second, and perhaps more important, is the fact that the sternite bristles occupy

a definite area, clearly delimited by the sternite itself, while the area occupied by the sternopleurals is somewhat variable and appears to have no clear-cut boundary. Thus, to the variation in density within a definite region, such as occurs in the sternites, is added the variation in extent of the potential bristle-forming region in the case of the sternopleurals. The latter type of variation might well be more sensitive to local conditions, and lead to the variation in bilateral asymmetry found in different genotypes.

There are good grounds, then, for maintaining the distinction between true environmental and chance variability in the case of the sternite counts, and of putting all the independent fraction of the variance in the latter class. This variability represents an aspect of developmental stability which appears to be very little affected by changing the genotype. But with the sternopleurals the distinction is not so clear, since some genotypes may produce correlated non-genetic variation on the two sides, while marked differences in asymmetry can occur between different genotypes. The nature of this variability clearly needs further study before we can decide how to classify it.

SUMMARY

1. Published data suggest that mean left-right asymmetry in number of sternopleural bristles of *D. melanogaster* declines when inbred lines are crossed, while the corresponding variance for sternite bristles remains unchanged. Some genetic tests were undertaken to analyse this difference in behaviour of the two characters.

2. A progeny test on a wild stock showed that a small amount of genetic variance in sternopleural asymmetry was present, equivalent to about 2% of the total phenotypic variance.

3. It was possible to increase and decrease the level of sternopleural asymmetry in two wild stocks by selection. These experiments gave an estimated heritability of some 2–3%, in close agreement with the progeny test. Change in asymmetry did not necessarily lead to a change in mean count.

4. Homozygous lines, consisting of individual third chromosomes from the Renfrew wild stock made homozygous in an inbred line genetic background, were intercrossed, and the average indices for a number of characters of eight intercrosses involving eight lines were compared with their mid-parent averages. Thorax length was 2% greater and its variance 32% less in the crosses; total sternopleural count and its variance did not change significantly, but the asymmetry variance declined by 18%. In contrast, the corresponding asymmetry or independent variance for numbers of sternite bristles was 6% higher in the crosses, although the total sternite count and its variance did not change. These results fit in with previous work.

5. Tests on a similar set of homozygous lines in which the third chromosomes came from the SP wild stock, and on some long inbred lines from the Pacific wild stock, gave discordant results. Of eight SP lines examined, four were homozygous

for a gene *polychaetoid*, and four were homozygous for a genetic effect causing sockets without bristles to occur among the sternopleurals. Both types had much greater sternopleural variance and asymmetry than the Renfrew lines, and both indices declined sharply in intercrosses leaving these genetic effects heterozygous, but neither declined if they were left homozygous in the crosses. Similarly high sternopleural variances were found in the Pacific lines, but only the total variance declined in males and only the asymmetry variance declined in the females, when they were intercrossed. All the four Pacific lines tested appeared to be homozygous for a genetic effect which caused a variable number of dorso-central and scutellar bristles to be replaced by sockets without bristles, and an occasional extra scutellar bristle to appear. This effect was also probably responsible for the high sternopleural variances.

6. Males of the Pacific inbred lines and intercrosses were compared when reared on the normal live medium and on a synthetic diet in reduced concentration, which reduced body-size by 23% (thorax area). The inbred lines were reduced more than the F_1's in total sternopleural count and its variance, but the F_1's were reduced more in sternopleural asymmetry, by the restricted diet.

7. The problems of interpreting these experiments, in view of our ignorance of the biological functions and attributes of the sternopleural and sternite bristles, are discussed. It is concluded that we have no basis yet for deciding whether sternopleural bristle number is of adaptive significance, but this is considered improbable.

8. The experimental evidence suggests that sternopleural asymmetry cannot be considered a measure of general developmental stability, particularly as the level of asymmetry can be reduced by selection well below that of typical wild stocks.

9. The scaling problems arising when the mean asymmetry of lines with different mean counts are to be compared, are examined, and it is suggested that the ratio of asymmetry to total count does not eliminate scale effects.

10. Developmental and anatomical differences between the sternopleural and sternite bristles suggest a possible reason why they behave differently when inbred lines are intercrossed.

REFERENCES

Auerbach, C. (1936). The development of the legs, wings and halteres in wild type and some mutant strains of *Drosophila melanogaster*. *Trans. roy. Soc. Edinb.* **58**, 787–815.

Clayton, G. A., Morris, J. A. & Robertson, A. (1957). An experimental check on quantitative genetical theory. I. The short-term responses to selection. *J. Genet.* **55**, 131–151.

Falconer, D. S. (1954). Asymmetrical responses in selection experiments. Symposium on genetics of population structure. *Int. Un. biol. Sci., Naples*, Ser. B, No. **15**, 16–41.

Harrison, B. J. (1954). Selection for differences in chaeta number between the fifth and fourth sternites of *D. Melanogaster*. *Drosophila Inform. Serv.*, **28**, 122–123.

Lerner, I. M. (1954). *Genetic Homeostasis*. Oliver & Boyd, Edinburgh.

Mather, K. (1953). Genetical control of stability in development. *Heredity*, **7**, 297–336.

Reeve, E. C. R. & Robertson, F. W. (1953*a*). Studies in quantitative inheritance. II. Analysis of a strain of *Drosophila* selected for long wings. *J. Genet.* **51**, 276–316.

REEVE, E. C. R. & ROBERTSON, F. W. (1953*b*). Analysis of environmental variability in quantitative inheritance. *Nature, Lond.*, **171**, 874–876.

REEVE, E. C. R. & ROBERTSON, F. W. (1954). Studies in quantitative inheritance. VI. Sternite chaeta number in *Drosophila:* a metameric quantitative character. *Z. indukt. Abstamm.- u. VererbLehre*, **86**, 269–288.

ROBERTSON, F. W. (1957). Studies in quantitative inheritance. X. Genetic variation of ovary size in *Drosophila*. *J. Genet.* **55**, 410–427.

ROBERTSON, F. W. & REEVE, E. C. R. (1955). Studies in quantitative inheritance. VIII. Further analysis of heterosis in crosses between inbred lines of *Drosophila melanogaster*. *Z. indukt. Abstamm.- u. VererbLehre*, **86**, 439–458.

SANG, J. H. (1956). The quantitative nutritional requirements of *Drosophila melanogaster*. *J. exp. Biol.* **33**, 45–72.

THODAY, J. M. (1958). Homeostasis in a selection experiment. *Heredity*, **12**, 401–415.

WADDINGTON, C. H. (1953). Epigenetics and evolution. *Symp. Soc. exp. Biol.* **7**, 186–199.

WADDINGTON, C. H., GRABER, H. & WOOLF, B. (1957). Iso-alleles and response to selection. *J. Genet.* **55**, 246–250.

WRIGHT, S. (1952). The genetics of quantitative variability. In *Quantitative Inheritance*, eds. E. C. R. Reeve & C. H. Waddington, pp. 5–41. Her Majesty's Stationery Office, London.

ity in

. VI.
ndukt.

ion of

VIII.
gaster.

gaster.

5.
199.
ection.

itance,
Office,